BOSTON UNIVERSITY
GRADUATE SCHOOL

Dissertation

THE METABOLISM OF ESTROGEN.

by

Joseph Schiller

(A.B.,University of Paris,1927 ;M.D.,Faculty of Medicine of Paris,1935;Licencié ès Sciences,Sorbonne,1936).

Submitted in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy
1944.

16

PhD
1944
S
copy 1

Approved

by

First Reader Leland C. Wyman
Professor of Physiology

Second Reader Burnham S. Walker
Professor of Biochemistry

Aknowledgements.

The author is indebted to Dr.L.C.Wyman for the keen interest he took in this work and for his constructive criticism.

To Dr.B.S.Walker for his many suggestions and useful indication of references.

To Dr.C.tum Suden for devoting precious time to the discussion of our problems.

To Dr.R.Crafts for making available animals from his special inbred colony.

Digitized by the Internet Archive
in 2016 with funding from
Boston Library Consortium Member Libraries

https://archive.org/details/metabolismofestr00schi

Contents.

I. Introduction _ _ _ _ i

Classical conception of hormones _ _ _ _ i

Present day problems resulting from recent investigation _ _ ii

II. The Estrogens _ _ _ _ 1

Main chemical and physical properties _ _ _ _ 2

III. Methods _ _ _ _ 4

Recovery of estrogenic substances from body fluids and organs _ _ _ 4

Urinary extraction _ _ _ _ 4

Extraction of estrogens from a perfusing medium _ _ _ 7

Extraction of estrogen from liver _ _ _ _ 7

Bioassay _ _ _ _ 8

Preparation of organs for perfusion experiments _ _ _ 9

Liver _ _ _ _ 10

Kidney _ _ _ _ 11

Heart _ _ _ _ 11

Hepatectomy _ _ _ _ 12

Ovariectomy _ _ _ _ 13

Adrenalectomy and adrenal transplantation _ _ _ _ 13

Perfusion pump _ _ _ _ 13

IV. Review of literature _ _ _ _ 18

Occurrence of estrogenic substances in nature _ _ _ 19

Occurrence of estrogenic substances in the mammalian body _ _ _ 21

Anabolism of estrogens _ _ _ _ 23

Ovaries _ _ _ _ 23

Testes _ _ _ _ 25

Adrenals 25
Placenta 29
Amount of estrogens produced in the body 31
Catabolism of estrogens 33
Humans 34
Chimpanzee 38
Equidae 41
Other species 41
The metabolism of exogenous estrogens 42
Results 43
Humans 43
Monkeys 45
Dogs 46
Rabbits 47
Guinea-pigs 49
Rats 49
Conclusions 50
Inactivation of estrogens by the liver 53
V. Experimental 59
Estrogens produced by the adrenal glands 60
The problem 60

Effect of adrenocorticotrophic hormone stimulation in:

    normal rat 62
    hepatectomized rat 62
    controls 63

Effect of transplantation of the adrenal glands into the spleen _ _ 63
Discussion _ 64
Conclusions _ 72
The fate of injected estrone in the adult male rat _ _ _ 73
The problem _ 73
Effects on normal injected rats _ _ _ _ _ _ _ _ _ _ _ _ _ 74
Effects on partially hepatectomized rats _ _ _ _ _ _ _ _ _ 75
Discussion _ 76
Conclusions _ 86
The perfusion of rat organs *in vitro* with estrogens _ _ 87
Liver perfusion _ 89
Kidney perfusion _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 90
Heart perfusion _ 91
Liver content of estrogenic substances _ _ _ _ _ _ _ _ _ 91
Discussion _ 92
Conclusions _ 100
VI. General discussion _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 103
VII. Bibliography _ 109

List of illustrations and tables.

Fig.1. The liver of the rat_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _16

Fig.2. Perfusion pump_ _17

Table I. Sources of estrogens_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _40

Table II.Effect of adrenocorticotropic hormone on the vaginal smear_ _ _ _ _ 70

Table III.Variations in the vaginal smear in partially hepatectomized rats_ _ _71

Table IV.The urinary estrogen of normal and hepatectomized male rats_ _ _85

Table V. The calculated recoveries of injected estrone_ _ 85

Table VI.The recovery of estrogenic activity from the perfusing medium_ _ _ _ _99

Table VII.The estrogen content of control and experimental rat livers_ _ _ _ _102

I. INTRODUCTION.

The conception of the interrelationship between the animal body and estrogens was largely inspired by the classical ideas of specificity. According to these ideas,each endocrine gland has a definite function which is performed through a specific chemical messenger. Only definite organs were considered as effectors to the exclusion of others. Structural, physiological and chemical specificity constituted a triad, the acceptance of which was largely justified by the progress made in the physiology of such glands as the thyroid,pancreas and adrenal medulla. However,step by step,physiological and chemical evidence accumulated challenging the idea of specificity or at least broadening it to such an extent that it lost its initial meaning. It was the physiology of the steroid hormones that became the turning point around which the pattern of thinking in endocrinology changed and undoubtely, the sex hormones played the largest part. For this purpose, the older techniques based on morphology or using the small amounts of estrogenic substances then available were unsatisfactory. Three fundamental discoveries have permitted the development of the newer knowledge of the physiology of estrogens during recent years:

1) The vaginal smear method bearing the name of Allen and Doisy(1) based on previous investigations of Stockard and Papanicolaou on the vaginal changes

in the guinea pig during the estrous cycle.(2) they extended it to other rodents such as the mouse and the rat thus making possible an estimation of both qualitative and quantitative activity of estrogenic substances.

2) The discovery by Aschheim and Zondek (3) that the urines of pregnant women, of pregnant mares and of stallions (Zondek (4) contained large amounts of estrogens thus making available new sources which could be used by biochemists for extraction and identification.

3) The isolation by biochemists of an estrogenic substance in crystalline form (Doisy et. al. 5), (Butenand 6) of known chemical structure which they returned to the physiologists for future investigation of the physiological activity.

Other discoveries of minor importance have greatly promoted recent advances. Among these special mention should be made of hydrolysis (Cohen et. al. 7), and the use of keto-reagents (Girard 8). Also more knowledge of the conditions under which certain physiological techniques are best employed has been obtained: organ culture (Carrel 9), transplantation of the adrenals (Wyman & tum Suden 10, 11) and other endocrine glands. In other words, it can be stated that all improvements accomplished were functions of improved physiological and biochemical techniques.

As far as estrogens are concerned several new facts

appeared:

(1) The sources of estrogens in the body are multiple and the ovary is not exclusive in the productions of these hormones.

(2) The secondary sex characteristics are not the only effector organs. They are not even necessary for the utilization of estrogen since their ablation does not alter significantly the metabolism of estrogens.

(3) Organs which apparently are not affected by estrogens play a larger part in their metabolism than those affected specifically.

(4) When estrogens are affected by the body the changes involved differ from one species to another and within the same species according to age, sex and various physiological conditions;

(5) The regulatory mechanism of hormone activity is no longer satisfactorily expressed in terms of a pituitary-gonad relationship or relative proportions of various steroids but has to be broadened by taking into account new factors.

All these facts will be discussed in the following chapters in connection with the experimental work.

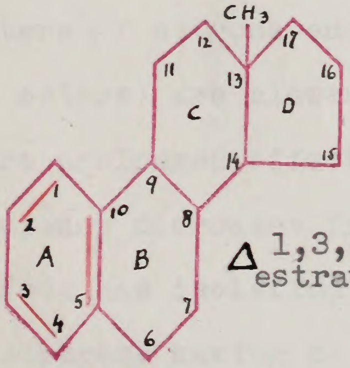
It is the purpose of this dissertation to further some aspects of the above mentioned phenomena in order to throw

more light upon the interrelationship organism -- estrogens.

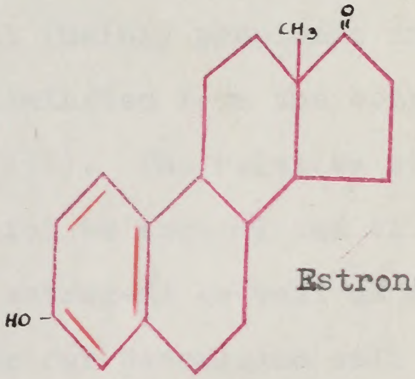
II. The Estrogens

The estrogenic substances are defined according to their ability to produce the typical changes of estrus in the genital tract of female rodents. This definition is physiological and independent of the chemical structure of the considered substance. For instance all synthetic substances with estrogenic activity obtained by Cook, Dodds, and their co-workers (87, 88) are chemically different from the natural estrogens. The latter are sterols (complex higher cyclic alcohols) related to cyclopentenophenanthrene and have four rings. When the rings are unsaturated at different linkages and provided with various side groups and side chains various steroids result: cholesterol, bile acids, sex hormones, organizer of the gastrula, etc.

If the first ring is unsaturated estratriene is obtained.



Δ 1,3,5-estratriene



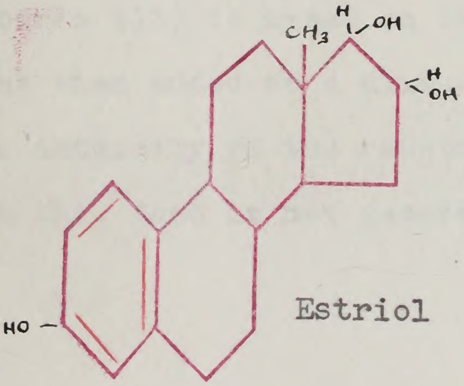
Estrone

Estrone differs from estratriene in that it has a hydroxyl group at carbon 3, and an atom of oxygen at carbon 17. It is a 3-hydroxy - 17 keto-estratriene.

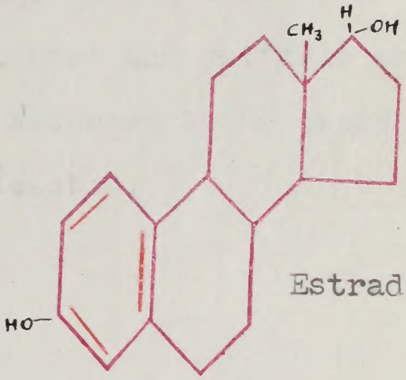
Estriol (its urinary form is estriol glycuronide) is a 3, 16, 17 - trihydroxy-estratriene.

The relationship of estriol to estrone has been established by converting the former to the latter by dehydration (Doisy) (12)

Estradiol is a 3, 17 - dehydroxy-estratriene and can be obtained by reduction of estrone in two isomeric forms A and B.



Estriol



Estradiol

Esters of estrone and estradiol (mainly propionic and benzoic esters) are slower to be eliminated from the body and give more prolonged effects(Doisy (12). The relative estrogenic potency decreases from estradiol to estrone and to estriol. The sources and isolation of these estrogens as well as of other estrogens having no bearing on our discussion will be described in Chapter IV Table I

Main Chemical and Physical Properties of the Natural Estrogens

All natural estrogens are phenolic and soluble in aqueous alkali. They are soluble in organic solvents such as ether, alcohol, acetone and relatively insoluble in petroleum ether

thus permitting their separation from other lipids.

In all estrogens ring A is aromatic and, consequently, the hydroxyl on carbon atom 3 is phenolic. The other point of substitution is at carbon atom 17 at which a carbonyl or a secondary alcohol is present. The phenolic hydroxyl forms esters with acids, acyl halides and acid anhydrides.

Colorimetric reactions have been described of which Kober's (13) is based on the change to a red color of estrogens when added to a mixture of phenol and sulfuric acid. The intensity of the reaction is supposed to be quantitative but this fact is not generally accepted.

III. METHODS

Recovery of Estrogens from Body Fluids and Organs

Urinary extraction

Hydrolysis and Extraction

The fact that urinary estrogens are usually excreted in conjugated form necessitates a method which would "free" them. This is insured by acid hydrolysis first used by Zondek (14) and thoroughly studied by Cohen and Marrian (7) and Smith (15).

The rat urines are acidified with concentrated HCl till the pH is brought down to 1-2. Instead of boiling as in the usual procedure with human urines the rodent urines are preferably autoclaved for 20 minutes at 14 lb. pressure. (Fish & Dorfman 16, 17, Heard et. al., 18)

The sample is cooled immediately and extracted by shaking in a separatory funnel with ether. The first ether represents about 20 per cent of the volume of acidified urine, the three others 12 per cent. The four ethers are combined and washed first with distilled water to remove the acid then twice with a saturated solution of sodium bicarbonate. The phenolic compounds are removed with 2 N NaOH (3 times) reacidified with concentrated HCl and cooled; again extracted four times with ether, washed with distilled water to neutrality and dried. This fraction represents the phenolic fraction containing the estrogens.

Fractionation

The dried sample containing all estrogens is fractionated

into "strong" phenolic (estriol), and "weak" phenolic ketonic (estrone) and non ketonic (estradiol). Estriol is first separated according to a method devised by Mather (20) in Doisy's laboratory and is based on the distribution coefficient for each of the above mentioned estrogens between immiscible solvents.

The residue containing the estrogens is taken in a volume of 25 c.c. of 0.3 Sodium Carbonate and extracted with an equal volume of benzene. Each fraction is reextracted with equal volumes of the opposite solvent. The two Na_2CO_3 washes are combined and acidified with 2 NHCl, extracted once with 40 c.c. of ether and two other times with 25 c.c. The three ethers are combined, washed with distilled water to neutrality and dried. This fraction contains the strong phenolic estrogen (estriol).

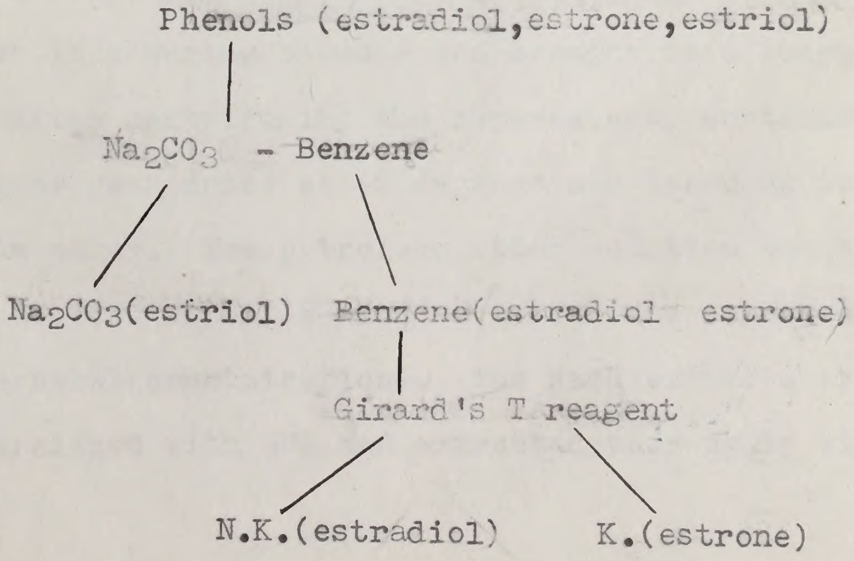
The benzene fraction containing the weak phenols is dried and left in a dessicator for several hours.

Partition

The separation of the non-ketonic (estradiol) from the ketonic (estrone) fraction is based on the use of the Girard's reagent (trimethylacetylhydrazide ammonium chloride) (Girard and Sandulesco 8). This reagent reacts with ketones to form derivatives which are soluble in water and insoluble in ether. The non-ketonic estrogens are not affected by this treatment and can be separated from the ketonic by ether extraction. After this separation the ketones can be liberated by hydrolysis and reextracted, the procedure is as follows:

The sample is transferred quantitatively to a flask with 10 c.c. of absolute ethanol and avoiding any moisture. The reagent (0.5 gm) is rapidly weighed and added to the flask together with 1 c.c. of glacial acetic acid. The flask is attached to a previously prepared reflux condensor and refluxed at a moderate rate for 90 minutes. After cooling it at room temperature, the solution is poured into 100 c.c. of ice water containing enough 10 per cent NaOH to neutralize exactly 0.9 of the used acetic acid. Extraction is performed at once with ether (3 times 50 to 100 c.c.) while keeping everything ice cold. The ethers are combined and washed with 25 c.c. of icy water which is added to the aqueous portion. The ethers are washed until neutral with distilled water and then evaporated. This fraction contains the non ketonic estrogens. The aqueous portion is acidified with concentrated HCl and let stand overnight at room temperature. The next day it is extracted with ether and neutralized as above and this fraction contains the ketonic estrogens.

The procedure is presented in the following scheme:


Phenols (estradiol,estrone,estriol)
Na_2CO_3 - Benzene
Na_2CO_3(estriol)
Benzene(estradiol estrone)
Girard's T reagent
N.K.(estradiol)
K.(estrone)

Extraction of estrogens from perfusing medium

In order to recover the estrogens from the perfusing medium an extraction method advised by Pearlman (personal communication) was employed. It was based on the addition of an alcohol-ether mixture (3 parts 95 per cent alcohol to one part ethyl-ether) using 18 c.c. of this mixture for each c.c. of blood or serum in the medium. The serum is added little by little, thoroughly mixed, heated gently to boiling and centrifuged until it becomes clear. The supernatant is removed and the precipitate reextracted with 4.5 c.c. of 95 per cent redistilled alcohol per c.c. of serum. New heating followed by centrifugation. The two samples are pooled together and evaporated. The residue is taken up in ether and extracted four times with N-NaOH acidified with 2N-HCl, reextracted with ether and neutralized. The fractionation of the various estrogens is performed as previously described.

Extraction of estrogens from liver

The extraction was accomplished by adding to each liver 15 c.c. of distilled water and 90 c.c. of acetone. The mixture was put in a Waring blendor and brought to a soupy consistency. After centrifuging the supernatant, containing the free estrogens, was dried at 40 degrees and taken up in 140 c.c. of petroleum ether. The petroleum ether solution was shaken twice with 35 c.c. of 0.1 N-NaOH to remove the estrogens (Mather, personal communication). The NaOH extracts are combined, neutralized with HCl and extracted thoroughly with

ethyl ether. This fraction contained the free estrogens.

To obtain the bound estrogens the precipitate from the aqueous acetone was taken up in 9 per cent NaCl and brought to pH 1 - 2 with HCl, hydrolyzed in the autoclave for 20 minutes at 14 lb. pressure and subsequently extracted as indicated above.

Animals

All rats used belonged either to the Sherman or to the Long-Evans strain. They were kept under conditions which are rather well standardized today as to food, drinking water, location, light, breeding, care of young, etc. (90)

Bioassay.

Bioassay was performed on spayed adult female rats according to the principles established by Allen and Doisy (1) and Long and Evans (21).

All animals were injected every five days, the average duration of their estrous cycle. The material to be assayed was injected in an oily solution subcutaneously in three doses, once every four hours. The newly spayed animals first received priming injections of a standard solution of estrone and in an amount over one unit in order to produce a full estrus. The few animals that showed negative smears were discarded and only those reacting positively were used for the assay of unknown solutions. The smears were taken 48 hours after the first injection, the desquamate being spread on a slide and a drop of water (or saline) added. Smears showing

epithelial or cornified cells were considered as positive.

Sixteen animals were used for each sample. The smallest amount which produced positive vaginal estrus in 50 per cent of the animals was considered as the rat unit. The results were plotted against standard curves established for estradiol, estrone and estriol according to the rules as established by Emmens (22) and applied by Pincus (23). According to these curves the r.u. for α-estradiol = o.125 γ, for estrone 1 γ, for estriol 1 γ.

Preparation of organs for perfusion experiments

The general rules for preparation of organs or anatomical regions for perfusion have been established by Carrel. They are:

1) sterile operatory technique,
2) integrity of vessels,
3) avoidance of embolus and thrombosis by air, blood clot or otherwise.

The sterile conditions are assured by autoclaving all surgical instruments, gloves and solutions for 1 hour at 126 C, and by circumscribing the operating field with pads soaked in Dakin's solution.

Two sets of instruments are prepared. The first set is used for the skin and muscles and exposing the organ (or anatomical region); the second set is used for removing the organ (or anatomical region). All instruments of the second set: scissors, dissecting forceps, artery forceps, are the

type used in blood-vessel or eye surgery.

Ether anesthesia was used in all cases. The animal was placed on its back with legs fixed in extended position.

Liver preparations

The hair of the abdominal area is removed with sodium sulfide and the skin cleaned with alcohol and iodine. A triangular incision is made in the skin, the apex at the vaginal papilla and the basis on a line connecting the lateral sides under the ribs. The aponeurosis is separated and cut together with the muscles according to the same lines as the skin incision. A forceps is applied to the flap and the abdominal cavity exposed. The intestine is displaced to the left of the operator and the peritoneum separated by blunt dissection along the abdominal aorta. The bile duct or the hepatic artery is cut (in the latter case below a ligature) and a needle fitted to a syringe filled with sterile Locke's solution (37 C) is inserted in the opperated vessel. An incision is made on the lower part of the abdominal aorta and the animal bled as thoroughly as possible. The immediate next step is the washing ofthe organ with the syringe already in place. This step is of utmost importance since thrombosis will be thus prevented. The change in color of the liver indicates the thoroughness of the washing. Immediately after the organ is separated from its attachments and care is taken to conserve as much as possible of the portal vein. The liberated organ is transferred to a sterile Petri dish containing warm Locke's solution and a canula of adequate size

and filled with the same solution inserted into the portal vein.

While the sterile perfusion pump is fixed in the perfusion chamber the prepared organ is kept in an incubator at 37 C. The fixing of the pump and of the perfusing medium as well as the canula with the organ is done within 10 minutes.

Kidney

The steps up to the exposure of the abdominal cavity are the same as for the liver. The difference in operatory procedure begins with the evisceration of the stomach, intestine and spleen. The peritoneum is cut progressively from the rectum up to the cardia and each vessel is cut between two ligatures. A thread is placed around the aorta below the origin of the renal arteries and the animal bled through an incision below the thread. The ligature is tied and a second incision made above the mesenteric artery, a syringe filled with Locke's solution introduced and the organs washed. When the change in color indicates the completeness of the washing, both kidneys are removed in a block with the corresponding segment of the aorta, and placed in a Petri dish with warm (37 C) Locke's solution. Ligatures are placed between the adrenals and the kidneys for the subsequent separation of these organs. The aorta is canulated through the proximal end, the distal being tied and fixed to the pump. The veins are left open to insure the return circulation.

Heart

The ribs are cut and the thorax opened; ligatures are put

around the vessels originating from the arch of the aorta. The vessel is cut, the animal bled and the heart washed. Canulation through the aorta and circulation insured through the caronary vessels.

The viability of the organs was estimated by microscopic examination of the sliced organ at the end of the experiment and in prolonged perfusions (10 hours) by histological examination.

Blood

Whenever rat blood was used several animals were bled through the abdominal aorta, the blood collected under sterile conditions and defibunated by shaking with glass rods.

Rabbit serum was mostly used because easily obtainable on the market in quantities such that it was not necessary to add Ringer Locke's solution as when rat blood was the perfusing medium.

Hepatectomy

This operation was performed according to the methods of Higgins and Anderson (24) and Selye (25) with a few modifications.

Anesthesia and cleaning of the skin was performed as previously described. An incision parallel to the right ribs and about ½ an inch below was made to the midline, the ribs slightly retracted and the liver exposed. The median and left lobes ligated (Fig. 1) with a black silk thread and extripated. Care was taken not to wound the bile duct,

hepatic artery and diaphragm. The wound was sewn and collodion applied. For the three following days 2 - 3 c.c. of an isotonic solution of glucose was injected intraperitoneally. The mortality was of about 20 - 25 per cent and the first post-operative 24 hours were the most critical. On the second day the animals already behaved normally.

Ovariectomy

A dorsal incision of the skin was made on the ether anesthetized animals followed by bilateral muscular incisions. The ovary with its fatty capsule was pulled out and cut below a ligature placed around short of the vistal end of the uterus. In this way all probabilities of regeneration were eliminated as shown by the control of the vaginal smear.

Adrenalectomy and adrenal grafting on the spleen.

A dorsal incision was made followed by bilateral incisions through the muscular wall. The right adrenal was freed first, the left second. The spleen was exposed through the same left incision and the two glands sewn around it. After the operation the animals were kept according to the rules established by Wyman and tum Suden (10, 11) The special drinking solution was not discarded before six weeks after the operation. The operative mortality was negligable and it is my impression that the spleen can share with the ovary the first place as a favorable location for adrenal grafting.

Perfusion pump (Fig. 2)

The construction of the perfusion pump is such that a

sterile pulsating circulation of fluid is maintained through the organ in order to keep it alive. It is made of pyrex glass and composed of two parts:

(1) the organ chamber,

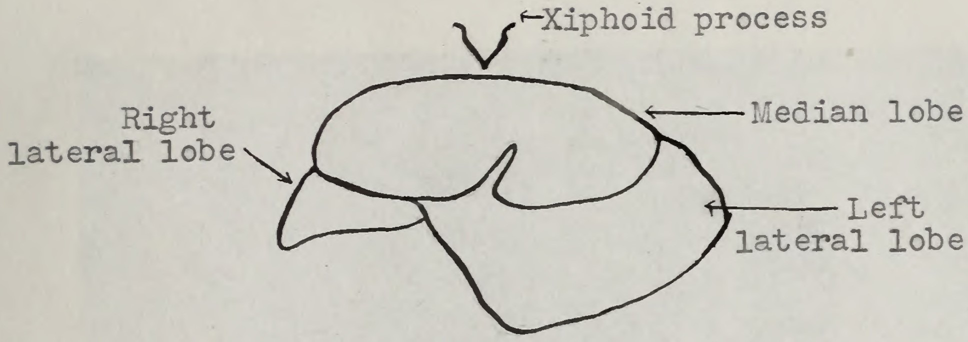
(2) the perfusing fluid chamber (reservoir).

It communicates constantly with the exterior through five openings, of which only three are used during the perfusion. The organ as well as the fluid never come in contact with any part of the apparatus communicating with the exterior. Those parts which do come in contact with the exterior are isolated by filter bulbs with non absorbent cotton. Fluid comes through the artery by pulsating gas pressure. The reservoir is connected by pressure tubing to a water column which is pulsated by a compressor valve system delivering 70 - 72 beats per minute. The liquid column stirs a column of gas which passes back and forth through the cotton filter bulb on the reservoir and causes the perfusion fluid to circulate. Positive pressure fits the glass valve of the inlet tube forcing the medium through the canula. When the positive pressure is cut off the glass valve of the inlet tube becomes seated preventing back flow, and the valve of the outlet tube lifts allowing the exit of venous perfusate into the reservoir.

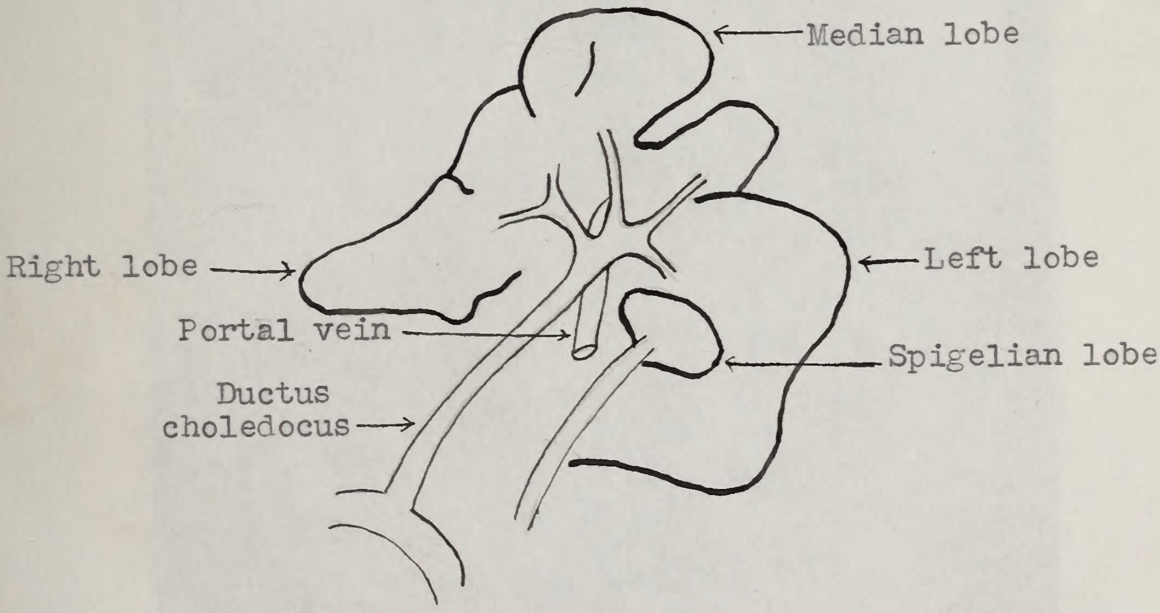
Humified air which enters the organ chamber under slight positive pressure, is mixed with the perfusate as a result of the ram action of the outlet tube.

The pump ensures a pressure of 120 mm. Hg. but variations can be introduced according to the experiment. The glass perfusion apparatus and the humidifying column are kept in an incubator at 37 C.

Fig.1. The liver of the rat.

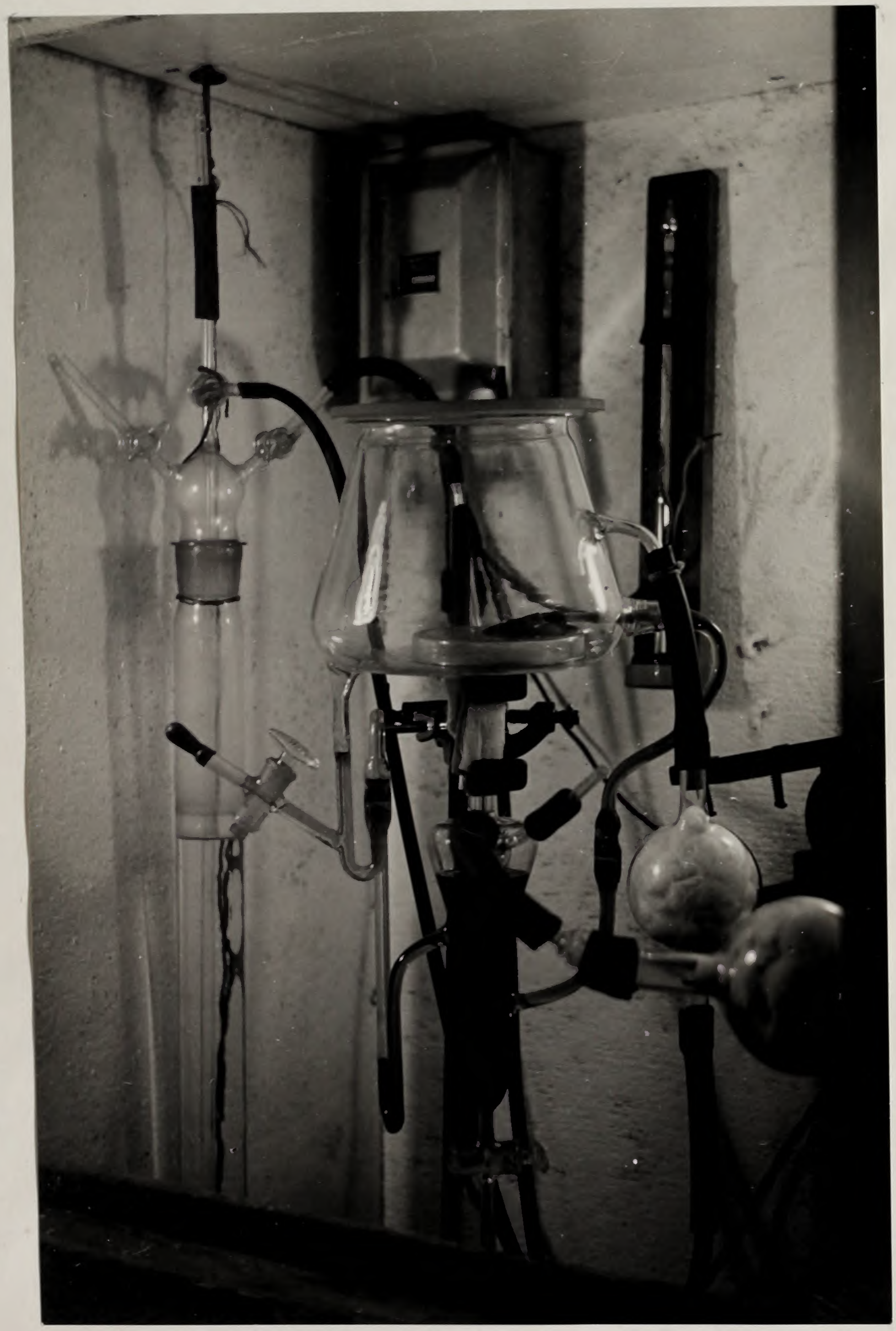

Xiphoid process
Right lateral lobe
Median lobe
Left lateral lobe

Ventral


Median lobe
Right lobe
Left lobe
Portal vein
Spigelian lobe
Ductus choledocus

Inferior

Fig.2. Perfusion pump.



IV. Review of Literature

Occurence of substances with estrogenic activity in Nature

One of the most unexpected results of the systematic investigation of substances with estrogenic activity was the widespread occurrence in the mineral, vegetal and animal kingdoms. In all cases, rough extracts were tested in ovariectomized mice or rats. A few examples will show that any attempt to correlate estrogenic activity with taxonomy is illusive.

Zondek (91) found that the sandy mud of the Dead Sea has an estrogenic activity of 100 m.u. per liter, while the surface water has none.

In plants besides the known **auxins**, Aschheim and Hohlweg (92) extracted estrogenic substances from various flowers but no attempt to identify them was made.

Butenandt and Jacobi (93) succeeded in isolating 18 mg. of pure estrone from 50 kg. of palm **Kernel** extract. Skargyuski (94) obtained 7.5 mg. of crystallized estriol from 65 kg. of willow female flowers.

In the animal kingdom estrogenic substances were found wherever systematic investigation was carried out. Steidle and Bauer (95) detected them in the **Infusorian** Colpoda Steini.

Steidle (96) found them in Echinoderms, Nematodes, Cestodes, Oligochetes and Molluscs.

Steidle (97) obtained similar results by using bees, spiders and a scorpion.

Donahue and Jennings (98) found that the estrogenic potency of the ovaries of Lytechinus variegatus was of the order of 1 r.u. per 4 gm. of dried ovarian tissue.

Loewe et. al., (99) claim that the organs of the moth Altacus athas contain as much estrogen as the corresponding organs of mammals i.e., 1 m.u. per 15 - 30 gm. of fresh ovary.

The fundamental difference in the secondary sex characteristics of the vertebrates and invertebrates is that in the former they are dependent on the gonads and their hormones while in the latter they are not.

The classical experiments of Goldsmith and of Morgan and his collaborators are in favor of a genetic determination of sex in invertebrates. Under such circumstances it is more cautious to assume that in invertebrates hormones are by-products of the general metabolism and this is substantiated by similar facts of the biology of invertebrates. (Delage 100, Smith 101). It is only in vertebrates that the estrogenic substances acquire more specificity. In other words estrogens existed before estrus. The only examples of a more specific influence of hormones in invertebrates are those of the corpora allata in insects (97) and of the regulation of chromatophore activity in crustaceans (176). This is not without analogy in the phylogenic development of other structures and functions following the same pattern throughout the animal kingdom. For instance, the antagonistic system, adrenalin-acetylcholin, is known to exist in all animal groups from the Protozoa up.

No anatomical substrata can be found and so far no definite physiological function could be attributed to them in lower forms. Also many pituitary functions exist in lower forms before the appearance of the gland or of any effector organ. (Gorbman 102).

Similar views are expressed by Lutz and Wyman (103) as a result of their investigations on the carotid sinus in Elasmobranchs. Such phenomena can be explained only in terms of evolution.

Occurrence of estrogens in the mammalian body

The search for natural estrogens in various organs and tissues of mammals other than those concerned in reproduction has been the object of only scattered investigations. Besides the improbability that such organs might produce estrogens technical difficulties are involved in their extraction. For this reason even in successful attempts caution is necessary in interpreting the results obtained. However, the enumeration of such organs furnishes some interesting data chiefly in connection with a possible storage of estrogens in the body and its ways of excretion.

Brouha and Simonnet (26) detected substances with estrogenic activity in the blood, uterus, mammary gland and milk of various mammals. Frank, Goldberger, Salmon and Friedman (27) found estrogenic substances in the psoas muscle of normal women as well as in an intermenstrual uterus. Trancu-Rainer (28) showed that the saliva of pregnant women is a means of

estrogen excretion.

Callow and Parker (29) while investigating the role of cattle testes and adrenals in the production of estrin and progestin-like substances, in addition showed that lipoid extracts from pituitary glands are estrogenic. This finding is interesting in the light of the physiological and cytological changes induced by exogenous estrogens on the pituitary gland. It is certainly not a by-product of the hypophysis itself but the result of a process of storage.

Fisher, Krohn and Zuckerman (30) extracted estrin from the sexual skins and exudates of 4 out of 14 monkeys under investigation. The interesting fact is that for the first time estrogenic substances were adequately extracted from a tissue on which they act specifically. What is the connection between the carcinogenic role of estrogens and the various tissues affected can be actually only a matter of speculation.

The role of urine, bile and blood as one of estrogen carriers is discussed in other chapters.

It is well known that estrogens are especially growth promoting substances and as such able to influence most of the tissues in plants and animals. (Zuckerman 31). But in order to classify an organ as an endocrine gland some definite conditions must be fulfilled.

Cytologically, secretion granules and an active Golgi apparatus should be present.

Physiologically the extirpation of the supposed gland

has to be followed by definite deficiencies that are eliminated by grafting the same type of gland. Instead of the graft its extracted product can be used as a substitute.

Chemically the extract has a definite structure.

Historically the first evidence came from clinical observations which support the preceding requirements.

None of these is satisfied by the above mentioned organs and consequently they can be excluded as playing any part in the production of estrogens.

Anabolism of estrogens

The following organs respond to all the conditions required for classification as endocrine organs with estrogenic function.

Ovaries

The ovaries are the longest known estrogen secreting organs and many aspects of their physiology are classical today and do not need to be discussed here.

In recent years the efforts of investigators have been centered around two problems. The first problem is of a cytological nature and attempted to establish the type of cell to which is devoted the production of estrogen. The various types of experiments devised attempted to determine the type of cell (germinal, granular, thecal) conditioning the development of the secondary sex characteristics. All evidence points toward the interstitial cell as playing an exclusive part.

(Geuther 32, Benoit 33, Schmidt 34, Corner 35).

The second problem has a more direct bearing on our discussion and is concerned with the isolation of estrogens from the ovaries. This phase of research is the result of the outstanding work of Doisy and his school.

Allen, Doisy *et al.*, (36) and Allen, Pratt and Doisy (37) took advantage of the fact that pig ovary follicles protrude and 6 to 7 ripen at the same time in each ovary. They extracted more than 2000 r.u. per kg. of tissue i.e., 0.5 c.c. of liquor folliculi was found to contain enough hormone to give a positive smear. The concentration of the hormone increased with the growth of the follicle. Sheep, cow, hen and human ovaries were subjected to similar procedures and gave similar results.

MacCorquodale, Thayer and Doisy (38) processed four tons of sow ovaries and recovered 400 liters of liquor folliculi which yielded 25 mg. of dehydrotheelin. They established that estradiol is the principal estrogen formed in the ovaries and identified it with the dehydrotheelin already prepared in the laboratory. The concentration in the liquor folliculi was about 1/15,000,000 and in the ovaries 1/150,000,000.

Westerfeld, Thayer, MacCorquodale and Doisy (39) found 20 r.u. of ketonic and 220 r.u. of non-ketonic activity per kg. of whole sow ovaries i.e., 0.010 mg. of theelin and 0.014 mg. of dehydrotheelin per kg. of tissue.

Testes

The presence of estrone in the urine of men has been known since the work of Laqueur (40) but its source was ignored. Surprisingly enough in view of the idea of internal gland specificity was the discovery by Zondek (4) that the stallion excretes in the urine 10,000 m.u. per liter i.e., 62 mg. a year. Immature or castrated stallions excrete only small amounts of estrogens. From the 350 gm. of testes tissue Zondek (4) obtained an extract containing over 23, 000 I.U. of estrogenic material which is 300 times as great as the content of both ovaries of a sexually mature mare.

In spite of large individual variations to which the excretion of estrogens is subjected in stallions (**Deulofeu** and Ferrari, **Haus**sler cited by Doisy (41) the testes of the stallion are still the richest source of estrogens.

Cunningham, May and Gordon (42) detected estrogenic substances in bull and deer testes.

Beale (43) succeeded in isolating both estrone and α-estradiol from horse testes with a yield of respectively 0.36 mg. and 0.21 mg. per kg. of tissue. For the first time was α-estradiol isolated from tissues other than of female origin.

Adrenals

The role of the adrenal cortex in the production and metabolism of all steroid hormones has achieved such importance in recent years that it deserves a large place in our discussion.

Besides the hormones influencing organic and inorganic metabolism in the body, other hormones from the androgen and progesterone groups have been identified. Therefore, it was not unexpected to find that the anabolism of estrogen was also affected by these endocrine glands. The relationship of the adrenal cortex to estrogens has various aspects that are reciprocal.

For instance, a synthetic substance such as desoxycorticosterone acetate has estrogenic activity (Salmon 44) while the estrogens have a corticomimetic effect on organic and inorganic metabolism (Long 45).

The estrogenic role of the adrenal cortex is the only one that interests us here and the evidence for it came from many fields of investigation.

Embryology

It is common knowledge that the gonads with the exception of the germinal cells are proliferated by the coelomic epithelium at the same time as the adrenal cortex and neighboring relations are maintained during the early embryological stages.

Histology

Goat (46) found adrenocortical-like tissue in the ovaries of adrenalectomized ground squirrels. Physiologically they behave like normal not like adrenalectomized animals.

Physiology

Wyman (47) showed that bilateral adrenalectomy interferes

with the normal estrous cycle in female albino rats. The maintenance of a normal cycle was correlated with some aberrant growth of cortical tissue.

Moon (48) has shown that in immature spayed female rats 22-23 days old injected intraperitoneally with adrenocorticorticotropic hormone the vagina opened earlier as compared with controls. The adrenals were found to be hypertrophied.

The converse experiment has been performed by Wade and Haslewood (49) who found that in ovariectomized-adrenalectomized rats the opening of the vagina is greatly delayed or even absent.

Bourne and Zuckerman (50) were able to demonstrate that a threshold dose of estrone that was not sufficient to maintain the endometrial growth in spayed adrenalectomized rats easily sufficed in reestablishing the estrous cycle in spayed non-adrenalectomized rats. The cyclic variations of the reacting endometrium were under the control of the adrenal cortex, the hypophysis being not responsible for it.

Pathology

Gardner (51) found tumors of the adrenal glands in 13 of 15 ovariectomized mice of the NH strain. The estrogenic activity of these tumors was indicated by the condition of the uteri, pelves and mammary glands.

The same result was obtained by Wooley, Fekete and Little (52) in the dilute brown strain. The castration of these mice brought a nodular hyperplasia of the adrenals accompanied by

changes typical of feminization as shown by the development of the uterus, vagina and mammary glands. The degree of feminization paralleled the degree of cortical hyperplasia.

In humans the adreno-genital syndrom is characterized by two types of manifestations: 1) masculinization in women with an increase in the output of androgens and eventually of estrogens and feminization in males.

The latter is the most interesting for us and its major clinical symptoms are: growth of the mammary glands, regression of the sex organs and loss of libido (Wintersteiner 53).

Frank (54) found in a case of adrenal carcinoma an excretion of 1000-10,000 m.u. of estrogenic subst.per day.

In a case of cortical malignant tumor (660 gm.) investigated as thoroughly as possible by Simpson and Joll (55) the output of estrogenic substances as determined by Burrow varied between 500 and 3000 m.u. per liter. The estrogen involved was in all probability estrone. The increase in estrogen excretion as revealed by the urine extracts paralleled the exacerbations of the patients symptoms. In a further analysis of this case Burrows, Cook, Rae and Warren (56) conducted several other investigations on the urinary extracts. Besides the estrone already mentioned growth promoting substances were found.

In order to detect compounds presumable with cortical-like activity the life maintenance test in adrenalectomized rats was used. The animals died after an average of 5.7 days as contrasted with controls subjected to cortin injections,

whose span of life was not affected. It resulted that the tested compound had no cortical activity in the administered doses.

Placenta

Half a century ago histologists already suspected an endocrine function of the placenta on account of their finding of secretory granules. However, it was only during the last years that this function has been substantiated by physiological and chemical evidence.

Allen (57) by means of bioassay in mice detected estrogens in the placenta and the chorionic membranes. Janney and Walker (89) obtained from placentas a minimum effective dose equivalent to 5 gm. of wet tissue when assayed in the mouse for estrogenic activity.

Allan and Dodds (58) investigated the case of a pregnant woman ovariectomized the 3rd month after conception. The pre-operative urinary output of estrogen was 2000 m.u. per liter as compared with 1400 m.u. per liter on the 2nd day and 1300 m.u. on the 62nd day after the operation. Though decreased the output was still high.

Probster (59) did not find a striking difference between the urinary estrogen excretion before and after ovariectomy in a 30 year old pregnant woman. A sudden fall occurred at delivery.

Hart and Cole (60) ovariectomized a mare at about the 200th day of pregnancy. After 144 days a normal foal was born

and normal lactation followed for 20 days when the mother was sacrificed. A marked fall in estrin excretion followed the operation for no plausible reason but during the last 60 days of pregnancy the content was comparable with that of normal mares. About 9,000,000 r.u. of estrin were excreted in the urine after ovariectomy.

Dorfman and Van Wagenen (61) took advantage of the fact that if the fetus is removed sometime between the 70th day of gestation the placenta remains in situ and is expelled at the time when delivery normally occurs. Parallel studies were made on 10 female Rhesus monkeys grouped as follows:

1. adult female monkeys;
2. pregnant female monkeys;
3. pregnant with the fetuses removed;
4. a pregnant monkey with ovariectomy and fetus removed in early gestation.

In all pregnant animals the excretion of estrogens amounted up to 46 times the non pregnant level. Conditions 3 and 4 did not interfere with the estrogen level maintenance as long as the placentas were retained. As in the case of human females the expulsion of the placenta coincided with a fall in urinary estrogens.

Chemical isolation in crystalline form has been performed for the three best known estrogens. Estrone was extracted by Westerfeld, MacCorquodale, Thayer and Doisy(62) who used 702 full term placentas (422 kg) with a yield of 20 per cent of the

extracts.

Huffman, Thayer and Doisy (63) obtained 0.038 mg. of -dehydrotheelin per kg. from the 422 kg. of placentas used. Estriol was isolated by Browne and Collip (cited by Doisy 41).

In conclusion it can be stated that the number of organs secreting estrogens is larger than formerly asserted. All have not the same functional value and the ovary is the most important. This organ as well as the adrenals (Bourne and Zuckerman, 50) have a cyclical feature. Others like the placenta are transitory. Finally under some pathological and experimental conditions the problem of estrogens of adrenal and testes origin is connected with that of intersexuality.

The chemical identity of estrogens being known the immediate problem is concerned with the amount produced under various physiological conditions.

Amount of estrogens produced in the body

There is no direct evidence concerning the amount of estrogens produced by the endocrine glands in the body. The only data available were obtained by estimating the amount necessary to induce certain known physiological phenomena in reacting organs and then to calculate the probable rate of secreted estrogens.

According to Corner (64) 125 I.U. of estrone injected daily for 10 days in the female Rhesus monkey will invariably result in menstruation-like bleeding in accordance with the estrin-deprivation theory of menstruation.

The converse experiment has been performed by Zuckerman (65) who found that with decrease of the daily injected dose of estrin from several thousand I.U. to less than 200 I.U. estrin-deprivation bleeding will occur in the next few weeks. From this sort of experiment it may be concluded that the daily output of estrogens in the adult Rhesus monkey would be somewhere between 150 - 200 I.U.

This result seems to find confirmation from another type of experiment (Corner, 64) in which the maintenance of the status quo of the Rhesus monkey uterus is brought about by using adequate doses of hormone. Under such conditions 125 I.U. daily will, in most cases, prevent post-castration bleeding in the female Rhesus monkey.

About 150 I.U. would be considered as the normal output if the maintenance of the color and edema of the sexual skin in the Rhesus monkey is to be taken as a criterion.

Results of the same order of magnitude were obtained in the Rhesus monkey by Hisaw and Greep (66) by injecting estradiol and progesterone in order to produce endometrial modifications.

Since the body weight in the human female is 15 times that of the female Rhesus monkey, Corner (64) calculated that the ovaries of the former must produce approximately 3000 I.U. daily.

According to Willard Allen (cited by Corner) (64) 4200 I.U. daily are necessary to produce bleeding in the ovariectomized

human female. Werner (67) agrees with this result.

Another method of determining the estrogen output in the human female is based on the calculation of the amount present in the urine during the menstrual cycle and then by injecting into the ovariectomized human female the necessary amount of estrogen that will reproduce the same urinary output. From the data of Koch (68), Smith, Smith and Pincus (69), Gustavson (70), Kemp and Pederson-Bjergaard (71) it results that 10 mg. of estrone is the normal output of which 1 mg. is excreted in the urine.

All investigators agree that these data are only approximations and ought to be accepted cautiously because of their indirect nature.

In a preliminary experiment Schiller (unpublished data) perfused the ovary of an adult female rabbit for 10 hours and obtained an output of 20 r.u. Calculated on a 24 hours basis the excretion of both ovaries would be of 100 r.u. The amount obtained was too small to permit a determination of the type of estrogen secreted.

Catabolism of Estrogen

The direct extraction of estrogenic substances from the producing organs is very laborious and costly. Besides that, the methods employed are not satisfactory for the physiological problems they propose to solve. Even when the site of production of estrogens could be located no further information could be obtained concerning the fate of the released estrogens in the body

in the body. Under such circumstances the epoch-making discovery by Aschheim and Zondek (3) of the great concentration of estrogenic substances in the urine of pregnant women offered new opportunities for research and, in fact, most fruitful ones.

The justification for using the analysis of urine estrogens is based on many arguments:

(1) The urine is the major route of elimination of estrogens by the body and can be readily approached technically speaking. The feces (Schubke 72, Dohrn 73) follow a parallel trend but other routes of excretion are unknown.

(2) Estrogens can be extracted in sufficient amounts for qualitative and quantitative determinations.

(3) The variations of estrogen excretion under various natural and experimental conditions can be followed daily.

(4) The urinary estrogens can be used as a test for practical purposes in clinical investigation of such cases as amenorrhea, sterility, menopause and others.

In Table I are recorded the principal estrogens isolated from various urines and in the following pages we shall discuss facts of physiological significance.

Human species

The metabolism of estrogens is a function of age and Webster (74) studied the estrogen and androgen excretion of 43 boys and 16 girls. A definite rise occurred at puberty.

According to Dingemanse (75) girls five years old excrete 50 I.U. of estrogenic substances per liter of urine.

In normal adult women Gustavson (76) found that the urinary estrogen excretion is cyclical and two typical peaks of yield were detected one toward the mid-cycle the other premenstrual.

Smith and Smith (77) found in four cases that the characteristic intermenstrual peak as measured by the total estrogens was lacking and assume that these were anovulatory cycles. Estriol reached its highest level during the luteal phase and practically disappeared during menstruation.

Smith, Smith and Pincus (69) in a thoroughly studied cycle of a normal woman found:

(1) more estrone than estriol was excreted during the cycle;

(2) during the first part of the cycle corresponding to the ripening of the follicle, the estriol level was low;

(3) the estriol level was higher during the luteal phase;

(4) the 48 hours preceding menstruation were characterized by a drop in both estrone and estriol levels, thus supporting the withdrawal theory of both estrogen and progestin;

(5) with the beginning of the flow the potency of the estrone fraction increased;

(6) the amount of estrogens excreted during a cycle equals 1 mg. of estrone.

From the above mentioned facts it may be concluded that:

(1) the ratio estrone/estriol varies with the various phases of the cycle;

(2) the determination of total estrogens is less important than the ratio of various estrogens;

(3) the appearance of progestin during the second part of the cycle affects the metabolism of estrogens.

In conclusion it can be said that the cycle is the result of a continuously changing hormonal equilibrium and this fact is even more obvious in pregnancy. During this condition the investigators found:

(1) an ascendency of estriol over estrone;

(2) the ratio estriol/estrone increases and supposedly coincides with the increasing activity of progestin;

(3) at parturition excretion of total estrogens is decreasing while the output of free estrogens increases;

(4) Estriol is the main estrogen excreted during pregnancy. In another experiment Smith and Smith showed that 80% of the estriol injected in pregnant women is recovered.

The two following points deserve attention because they reflect changes in the clinical condition of a pregnant woman:

(1) A drop in the estriol excretion coincided with a flow of blood showing a deficiency somewhere in the complex mechanism of pregnancy;

(2) when false labor occurred this was paralleled by

a rise in the estrone/estriol ratio.

The increase of urinary estrogen output up to 1000 times the normal excretion has been confirmed by Doisy (78). Biochemical and physiological investigations bring further evidence of the fact that different modalities of estrogen metabolism correspond to different physiological conditions.

Marker (79) extracted from the urine of pregnant women two estranediols but was unable to obtain them from non pregnant women.

Palmer (80) found that both normal and pathological uterine bleeding are associated with the presence of free estrogenic hormone in the urine. In the urine of normal women of reproductive age estrogens are combined. The daily excretion varies between 4 - 30 mg. of estrone, except during the 1-2 days of the midintermenstrum when it reaches a height of 200 yg.

Hain (81) also found that the last three weeks of gestation are characterized by a marked increase of combined estrogens and pregnanediol, the maximum excretion being attained generally eight days before parturition.

The initial stage of menopause is supposedly characterized by an increased excretion of estrogens (Sainton, 82). In men Pincus and Pearlman (83) found no cycle of urinary estrogen excretion and also that the titers of the various estrogen fractions is lower than in women.

Chimpanzee

Allen, Diddle, Burford and Elder (84) found that the urinary estrogenic output at various stages of pregnancy varied between 170 - 620 m.u./liter of urine. In normal females the output varied from 79 - 172 m.u./24 h. with a peak at the mid-cycle and the lowest yield during menstruation. The urinary variations parallel the swelling of the external genitalia. Fish, Young and Dorfman (85) recovered from the urine of a chimpanzee female 10 I.U./24 h. during menstruation and 170 I.U. at the time of maximum genital swelling which occurred at the 15th day of the cycle. A second premenstrual peak was reached the 25th day followed by a new decrease toward the next menstruation. In a second case while the same amount of estrogens was recovered as in the first case the peak reached a value of 385 I.U. thus showing individual variations.

These data confirm Allen's finding concerning the high value of estrogen output during the maximum genital swelling and the low estrogenic output during menstruation. They differ, however, inasmuch as Allen does not detect the second peak of the cycle. As a whole the pattern of the cycle in the female chimpanzee is perfectly parallel to the one found in human females. Another interesting remark is that the urinary estrogenic output in female chimpanzees occupies, as do many other anatomical and physiological characters, an intermediate position between the human female and the Rhesus monkey female. The latter primate has been investigated, in this respect,

by Allen, Maddus and Kennedy (86). In male chimpanzees the average output is 70 I.U. of estrogenic substances per day.

Table I.

Species	Estrogen	Source	Investigator
Human.	Estrone	Pregnancy urine	(Doisy,Veler,Thayer 1929; Butenandt 1929.)
		Male urine	(Dingemanse,Laqueur and Muhlbock 1938).
	Estradiol	Pregnancy urine	(Smith,Smith,Huffman,Mac Corquodale,Thayer and Doisy 1939).
	Estriol	Pregnancy urine	(Marrian 1930;Doisy, Thayer,Levin and Curtis 1930).
	Estriol glucuronide	Pregnancy urine	(Cohen and Marrian 1935).
Horse.	Estrone	Mare pregnancy urine	(Laqueur 1931).
		Stallion urine	(Haussler 1934;Deulofen and Ferrari 1934).
	Estrone sulfate	Mare pregnancy urine	(Schachter and Marrian 1938).
	Equilin	Mare pregnancy urine	(Girard,Sandulesco, Friedenson and Rutgers 1932).
	Equilenin	Mare pregnancy urine	(Girard,Sandulesco, Friedenson and Rutgers 1932).
	Hippulin	Mare pregnancy urine	(Girard,Sandulesco, Friedenson and Rutgers 1932).
	α-estradiol	Mare pregnancy urine	(Wintersteiner,Schwenk and Whitman 1935)
	β-estradiol	Mare pregnancy urine	(Hirschman and Wintersteiner 1937).

Note. The urinary recoveries in other animal species were too small to allow the chemical identification of the natural excreted estrogens.

Equidae

In the case of horses it is of the highest interest to contrast on the one hand the presence of both estrone and estradiol in the testes with the recovery of only estrone from the urine of the stallion (Hanssler, 104) and on the other, the numerous estrogenic metabolites extracted from the urine of the pregnant mare. (Table I). According to Cartland et. al., (105) stallion urine is superior to that of the pregnant mare as a source of hormone because the estrone is relatively free from related substances. These investigators obtained 38,000 r.u./ liter of urine from which 16 mg. of crystalline estrone was obtained. The urine from a two year-old colt contained no estrogenic material.

Other species

Urinary estrogens have been detected in the urines of female rabbits (Beerstecher 106), pregnant female rats (D'Amour, Funk and Glendenning 107) immature rats of both sexes (Dorfman 108), female guinea pigs with irradiated ovaries (Schmidt 34) and bull urine (Marker 109)

Although in all these cases, urinary estrogens were demonstrable by bio-assay, the amounts recovered were too small to permit a complete study.

In other species (dog) no estrogenic substances could be detected in the urine (Longwell and McKee 110).

The results obtained from the study of qualitative and quantitative recoveries of urinary estrogens amply justifies le rats (D'Amour,

its use. It illustrates the fact that there is no one means of metabolic possibilities but"metabolisms"according to the species investigated and within the same species according to age,sex and physiological conditions. It also implies that the role of the effector organ is a major one.However,in spite of its many advantages,this method of investigation has its limitations since only end results are available.It does not give any available information about the site of production of estrogens and even more disturbing is the fact that none of the intermediary steps of the metabolism of estrogens can be detected nor the organs originating them.It can be said that the results obtained are at the "body-level" and of little analytical value because no links can be established between them. In extreme cases no data at all are available.Other methods of investigation,however,have been devised which permitted a step forward in solving the problem of metabolism of estrogen.

The Metabolism of Exogenous Estrogens.

The studies of endogenous estrogens,however interesting in themselves,do not supply enough information to elucidate the major problems involved in their metabolism.Neither the nature of the intermediary changes nor the extent to which they occur is understood. To obtain further information on these points the technique of injection of exogenous estrogens was resorted to because of its greater value.The basis of the technique is to inject definite amounts of an estrogen of known chemical structure and to extract from the urine the eventually occuring metabolites.This technique will necessarily furnish

more information on the catabolism of estrogens than on their anabolism.

Besides the species differences and various physiological conditions such as sex, age and integrity of various organs, with which we dealt in the study of endogenous estrogens new factors have to be considered with this technique.

(1) In order to isolate and identify biologically and chemically the urinary metabolites, more estrogen should be administered than the body can oxidize.

(2) The nature of the estrogen (free or ester), the concentration and nature of the solvent (oil, alcohol, water), the number of injections and their timing are all important, and differences in findings might be partly explained in terms of these variations.

(3) The same caution is necessary in the evaluation of extraction and isolation methods, which in spite of recent advances are not perfect, and the possibility that metabolites can be present though not actually detectable cannot be excluded.

Results

Humans

Smith and Smith (15) after injecting 50,000 I.U. of estrone along with 10 mg. of progesterone in a woman recovered in a 48 hour urine collection 16% of the injected material of which 42% was represented by estrone, 20 % by estriol and the remaining 38% by a product that, in all probability, was estradiol.

No experiment with estrone alone was performed.

Pincus and Pearlman (111) in a study of the urinary excretion of estrone injected into a group of female patients, non cancerous and with no apparent endocrine disease, as compared with a similar group of men obtained a total recovery of less than 2%, the estrone having been partly converted to estradiol and chiefly to estriol.

The same investigators (76) collected and pooled together the 96 hour urine collections of seven healthy young men who received a total of 1.05 of estrone acetate in oil. Of the 1,000,000 r.u. injected they recovered:

23,000 r.u. as estrone,
28,000 r.u. as α - estradiol,
28, 500 r.u. as estriol,

thus showing an extensive inactivation and the conversion of estrone to both α -estradiol and estriol.

The reverse conversion from α-estradiol to estrone has been obtained by Heard and Hoffman (112) who injected intramuscularly 250 mg. of α-estradiol into normal young man and collected the urine over a period of 8 days. The efficiency of the estrogen was demonstrated by symptoms such as transitory gynecomastia and nausea. Only 3.9% of the injected α - estradiol was recovered unchanged, while 6.4% was eliminated as estrone. Unfortunately for theoretical reasons these investigators discarded the possibility of a conversion to estriol, thus leaving this point unsolved.

Reconsidering the problem,Schiller and Pincus(113)injected intramuscularly into a normal human male 21.60 mg. of α-estradiol and recovered 0.99% as α-estradiol,2.68% as estrone and 5.42% as estriol,thus indicating that estriol is an end product of α-estradiol conversion in man.They further showed that estriol was convertible to more potent estrogen.Of 37.34 mg.of estriol diacetate injected into the same subject,56.57% was excreted as such without alteration of the normal output of estrone and α-estradiol.

That inactivation of estrogens does occur has been shown also by Kemp and Petersen-Bjergaard (114)who obtained in women a recovery of up to 10% of the injected estrogen. They did not consider the possibility of estrogen conversion however.

Approximatrly the same order of values was obtained by Mazer and Israel (115)

Monkeys

Frank,Goldberger and Spielman (116) reported a recovery of almost 20% of the administered estrogen.

Westerfeld and Doisy (117) give the following data:

(1) three mg. of dehydrotheelin over a 6 day period permitted a recovery of 3.8% in normal adult females, 1.5% in ovariectomized and 3.5% in hystero-ovariectomized animals,all data being calculated as dehydrotheelin.

(2) Six mg. of theelin daily over a 6 day period gave respectively recoveries of 2.7% in normal females,

1.97% in the ovariectomized ones and 5.3% in the hystero-ovariectomized, all data being calculated as theelin.

In both groups of experiments the conversion of theelin to dehydrotheelin was found to be reversible. The results indicated that the ovaries, the Fallopian tubes and the uterus are not necessary for either inactivation or conversion.

Marker and Hartman (118) after injecting 1.097 gm. of theelin subcutaneously into a pregnant Rhesus monkey over a 20-day period, starting the 78th day of pregnancy, could not isolate any estrone from the urines.

Doisy, Thayer and Van Bruggen (119) injected an aqueous suspension of β-estradiol and found strong indications of a conversion of this estrogen to α-estradiol after passing through the phase estrone.

Van Bruggen (120) injected 500 mg. of powdered estriol in aqueous solution into 2 monkeys over a 5-day period. About 7% was excreted in the urine but the duration of the excretion was prolonged for 20 days after the cessation of the injection. This result implies that estriol is more resistant to destruction than other estrogens. No conversion to estrone or estrtradiol could be detected.

Dogs

Kunde et. al. (121) gave 200 r.u. of a chemically undetermined estrogen in subcutaneous injection for 39 days.

The recovery averaged about 50% which, if correct, is higher than in any other species.

Stamler (122) injected intravenously 1000 m.u. per kilogram of weight in several dogs of both sexes and after collecting the urines by catheter found an excretion of up to 10.9%.

Dingemanse and Tyslowitz (123) recovered from 7.2 to 20% of the injected estrogen and besides that, proved that independently of the nature of the injected estrogen (estradiol or estrone) both fractions were represented in the urine with a ratio of ketonic to non-ketonic always in the favor of the latter.

Cantarow, Rakoff, Paschkis and Hansen (124) recovered 10% from the urine in the 24 hrs following the intravenous injection of 250,000 I.U. of estrone. The output decreased rapidly during the following days.

About the same amounts were found by Longwell and McKee (110) with a range in the urine from 6.4% to 9.7% of the injected estrone.

Rabbits

The first experiments with this animal were those of Smith and Smith (125) who, after injecting 600 - 700 r.u. of estrin, recovered from 22 to 67 r.u. Ovariectomy had no effect upon inactivation while the addition of progestin increased the output of estrogen tenfold.

It was, however, the work of Pincus and Zahl (126) that furnished the basis for further investigation of the

physiology of estrogens. After injecting 300 micrograms of estrone or estriol into rabbits in various physiological conditions such as heat, pregnancy, pseudopregnancy, ovariectomy and pseudopregnancy in heat, they formulated the following rules:

(1) estriol is not converted to estrone or estradiol;

(2) estrone is converted to estriol in animals with a functional uterus;

(3) estradiol is converted to estrone in the presende of ovaries.

While the principle of conversion has been repeatedly confirmed since, the importance of the uterus and ovaries in the metabolism of estrogens has not been generally accepted.

Heard, Bould and Hoffman (18) injected 300 mg. of α-estradiol into a pair of adult females and isolated the hormones from the urine in crystalline form. The β-estradiol excretion represented 12.1% as compared to 2.6% of estrone. In the same way the injection of estrone gave in the urine 4-5 times more B-estradiol than estrone. No estriol was obtained unless injected as such. Ovariectomy or hysterectomy did not alter the proportions of the excreted estrogens.

Fish and Dorfman (17) confirm the preceding data and stress the fact that α-estradiol is thoroughly metabolized in the rabbit with β-estradiol as the end product and estrone the intermediary.

Stroud (127) could detect only a non ketonic metabolite,

probably β-estradiol, but no estriol after the injection of estrone.

Van Bruggen (120) is the only investigator who, in preliminary experiments, has found indication that the rabbit converts B-estradiol to a ketonic estrogen and to estriol.

Guinea pigs

Fish and Dorfman (128, 16, 129) studied normal and ovariectomized females as well as males. Estradiol and estrone were given orally or subcutaneously and the occurring metabolites isolated as crystalline compounds from the urine. In all cases the recovery was less than 5% and the interconvertibility (estrone-estradiol) obvious. The conversions occurred in the absence of the ovaries.

Emmens (130) recovered up to 15% of the injected estrone.

Rats

In these rodents, the findings are conflicting as far as the amounts of recoveries are concerned. Zondek (14, 131) could not extract from the urine more than 0,6% of 20,000 m.u. of injected folliculin and no more than 1% from the total animal. However, after hydrolysis 20% was obtained 24 and 6% 48 hours after the injection. He established for the first time that the animal body does not store estrogens. No sex differences were noticed.

The data of Zondek were confirmed by Dingemanse and Laqueur (132) who recovered about 2% of the 25,000 r.u. injected.

Dorfman (108) succeeded in obtaining larger recoveries than the preceding investigators. The injection of 25 animals

micrograms of theelin in oil into sexually immature animals resulted in an urinary output of 10%.

In none of the investigations on rats has any attempt been made to identify the nature of the excreted estrogen.

Conclusions

The preceding survey of the metabolism of exogenous estrogens in various species of mammals clarifies a number of unknown points and permits some definite statements.

The first conclusion which includes all facts is that the exogenous estrogens are affected by the living body both quantitatively and qualitatively.

The most striking phenomenon is the intensity of the inactivation which in practically all cases represents over 80% of the injected amount . At the same time the pace of inactivation occurs at high speed, being a matter of a few days even when large quantities of estrogens were injected.

No less striking is the conversion, not only from the more potent estrogen, α-estradiol to the less potent estrone and estriol, but also from estrone to α-estradiol.

The general scheme proposed by Pincus and Zahl (126) remains true in the majority of cases:

$$\alpha\text{-estradiol} \rightleftharpoons \text{estrone}$$

as a reversible reaction.

However, species differences should not be overlooked as shown by several examples:

(1) In the humans the preceding scheme would be incomplete without the integration of estriol as an end-product according

to the following scheme:

$$\begin{tikzcd}
\text{estradiol} \arrow[rr, shift left] \arrow[dr] & & \text{estrone} \arrow[ll, shift left] \arrow[dl] \\
& \text{estriol} &
\end{tikzcd}$$

Estriol is not convertible to any other estrogen and is more resistant to inactivation as shown by its prolonged excretion in the urine and its more efficient oral activity as compared with either estradiol or estrone.

(2) In monkeys the interrelationship is

β-estradiol ⟶ estrone ⟶ α-estradiol

while the reverse is true in rabbits:

α-estradiol ⟶ estrone ⟶ β-estradiol.

These three examples emphasize the basic fact of the importance of the reacting organism rather than that of the injected estrogen. Both humans and guinea pigs interconvert α-estradiol and estrone but they differ in the production of estriol which does not occur in the latter.

It is obvious from all three examples that the same estrogen is metabolized differently in closely related animal species which is somewhat surprising in view of the unity of morphogenetic plan through all mammals.

As always occurs in scientific investigation the solution of a problem far from being an end is only the beginning of new problems. In view of the fact that presence of the ovaries of the Fallopian tubes and of the uterus is not necessary for either inactivation or conversion of estrogens our next

problems in the discussion of the metabolism of estrogens are: What are the organs involved in the inactivation and conversion of estrogens and what is the nature of the mechanism producing it?

Inactivation of estrogens by the liver

The results of hormone administration studies, aside from their intrinsic value, show neither the nature nor the direction of inactivation. Various experiments have been undertaken which intended to discover the organ or organs in which these phenomena can occur, and it is logical to assume that primary attention be given to the end-organs, ovaries, Fallopian tubes and the uterus. It soon became apparent, on the one hand, that these organs were not necessarily the most important and on the other hand that there were few organs and tissues which were not affected by the estrogens. The bulk of evidence points to the liver as playing the major role in the catabolism of estrogens. Golden and Severinghaus (133) homotransplanted rat ovaries into the mesentery. Controls received ovarian grafts in the axillary region. Both grew well but cornified vaginal smears occurred in controls only. When the ovaries were grafted from the mesentery in the axillary region normal vaginal smears were reestablished.

The idea of a loss of potency by estrogens has an empirical basis in the observation that exogenous estrogens administered orally had little effect on the body. The first experimental confirmation came from Evans and Buhr (134) who showed that whole pig-ovary extract has not its characteristic action when injected intraperitoneally. Following the work of these investigators, a large number of experiments, some

of qualitative value only, but some providing quantitative data have been conducted which lend support to their findings. Fee, Marrian, and Parks (135) by using the isolated heart-lung-kidney preparation without liver arrived at the conclusion that the active agent of destruction is oxygenation by the lungs. Silberstein and co-workers (136) by incubating mashed liver with estrogens discovered that they became inactivated and are the only investigators to claim the inactivating role of the blood. Talbot (137) destroyed the functional activity of the liver by injecting into immature female rats a mixture of CCl_4 and 95% ethyl alcohol, and thus enabled the endogenous estrogens to act upon the uterus. Nothing similar occurred in uninjected controls. This experiment indicates that the inactivating agent is the liver cell itself. Pincus and Martin (138) after establishing the lethal and sub-lethal doses of CCl_4 for rats, confirmed the role of the liver cell in the metabolism of exogenous estrogens but did not obtain evidence for the presence of endogenous extra-ovarian estrogens in spayed animals. That the reticulo-endothelial system of rats is not involved has been shown by Zondek and Sklow (139). Broadening the field by including various steroid hormones as well as artificial estrogens, Selye (25) used their property to produce anaesthesia in partially hypatectomized young rats to show that they are more sensitive than the controls. It may be concluded, therefore, that the liver plays a role in the detoxification of these substances. Biskind and Mark (140)

were unable to produce the estrus type of vaginal smear by implanting pellets of estrone into the spleen and the liver of castrated female rats. On the other hand, cornification occurred when the spleen was transplanted under the skin outside the area of the portal system. In the same way both estradiol and estradiol benzoate could be inactivated (Biskind, 141). Segaloff and Nelson (142) injected estrogens directly into the spleen of castrated female rats and found an almost complete destruction of them. As for humans Glass and coworkers (143) have brought clinical proof that liver cirrhosis, which is essentially a disease of the hepatic cell, is accompanied by abnormal excretion of estrogens in free form.

Zondek (14) was the first to bring more complete quantitative data. Using as a point of departure the evidence that very little of injected estrone is eliminated, he analyzed the extract of the whole animal and excluded the possibility of the body storing the hormone.Pursuing the problem further he found by incubating slices of various organs with estrone that the liver can produce a loss of activity as high as from 87.5 to 93.75%. In Stamler's (122) experiments with dogs more than 90% of the 1000 m.u. injected ~~it of folliculin~~ disappeared from the blood stream immediately after injection. In the bile the folliculin appeared within from 15 to 30 minutes, reached its peak at the end of the first hour, and disappeared at the end of the fourth hour. The liver of injected animals contained more folliculin than other organs. Israel and

co-workers (144) by using comparatively dog heart-lung-liver and heart-lung preparations found that the inactivation of 100 r.u. of estrone by the liver to be a matter of minutes. Heller (145) by incubating slices of various rat organs with 0.3γ of estradiol obtained, as far as the liver is concerned, a total inactivation and thought he had excluded any possibility of a conversion to the less potent estrogens estrone and estriol. Added estrone was also inactivated, estriol only slightly. Heller and Heller (146) found no difference between the organs of pregnant and non-pregnant animals. Twambly and Taylor (147) confirmed the estrogen inactivating role of rat liver slices and showed in addition that the livers of patients dying of cancer and those of mouse strains susceptible to mammary carcinoma do not differ essentially from those of rats with regard to this property. Dingemanse and Tyzlowiz 9123) discovered that dog's liver contains more estrogens than other organs in relation to its weight. It is interesting to note that in their experiments the ratio non-ketonic (N.K.) to ketonic estrogen (K.) in the urine is always in favor of the nonketonic.

The bile as an avenue of excretion of estrogens as compared with urinary excretion has been investigated by Longwell and McKee (110). After 72 hours 5 to 8% of 1 mg. of injected estrone was recovered from the bile, and all estrogens were in the non-ketonic form, while in the urine both non-ketonic and ketonic were present. That the normal bile does not con-

tain estrogens is confirmed by Cantarow and co-workers (148) who in opposition to Longwell and McKee recovered in 72 hours almost all of the 250,000 i.u. of estrone. Their conclusion is that the liver can store large amounts of estrogens and reveals doubt that rapid destruction or inactivation is possible in the intact animal. They assert the existence of an enterohepatic cycle, and bring supplementary evidence for it in the dog and pregnant woman with a decrease in the latter case in the post partum. In later work (149) they concluded that inactivation of estrogen by the liver was a relatively slow process. The estrogen being first converted to an inactive form in the liver and later being reactivated on its excretion in the bile. This claim has no other proponents.

The estrogen-inactivating action as a general property of liver tissue and independent of any species is shown by Engel (151), who discovered the same phenomenon in the guinea pig with a loss of potency of added estrone of from 33 to 66%. Segaloff (150) basing his research on the inactivation of estrogen by the rat liver tried to quantitate this inactivation and found it to be dependent upon the nature of the individual estrogen used. Biskind and Meyer (1943) by implanting pellets of estrone into the spleen of normal male rabbits found no effect on the testicles while subcutaneous implantation of similar pellets in control rabbits inhibited spermatogenesis. The independence of the liver's ability to inactivate estro-

gens with respect to homeo- or poikilothermia has been investigated by Zondek (14) in Rana esculenta and confirmed by Engel and Navratil (152).

The generality of inactivation in nature has been disclosed by Enler and Zondek's experiments (153) on the hyacinth. This plant inactivated 75 per cent of 200 m.u. of folliclin dissolved in 400 c.c. of water in a few hours.

The mechanism of inactivation is little known. Zondek (14) suggested the action of an enzyme "estrinase" as being the inactivating agent. Heller (145) supports this view and believes in the existence in the body of two mechanisms controlling the metabolism of estrogens: an oxidative and an overflow mechanism. The nature of the enzyme itself is problematical. Westerfeld (154) suggested that estrone and estradiol are inactivated by tyrosinase. Oxidation as a mechanism of inactivation in mammals is doubted by Graubard and Pincus (155), who were unable to obtain an oxidase from the liver and who attribute Westerfeld's results to the presence of laccase in his tyrosinase preparation. Zondek and Sklow (1942) reject the possibility of either one of them as being the specific plant enzyme.

The effect of a vitamin B complex deficiency as inhibiting the estrogen-inactivating mechanism of the liver is suggested by Biskind and Shelesnyak (156) and by Biskind and Biskind (157). The ability to inactivate is restored by brewers yeast.

V. EXPERIMENTAL

Estrogens Produced by the Adrenal Glands

The interrelationship between the adrenals and the ovaries is well established and finds a strong basis in the fact that their products are chemically closely related substances. Their activities are reciprocal and synergistic and influence both morphological and physiological manifestations in the body. To the corticomimetic action of estrogens upon the inorganic and organic metabolism (Thorn and Eugle 158; James and Nelson 159; Ingle 160; Long 45) corresponds an estrogen-like action of the adrenals. (Salmon 44)

There is a marked sex difference in the size of the adrenals which, in rodents, is heavier in females than in males (Cole and Harned)(161).

Castration induces hypertrophy of the zona fasciculata and reticularis in the male rat adrenal (Hall and Korenschevsky 163) as well as in the young female. The influence of the age factor in the latter case has been emphasized by Hashimoto (164).

More cortical hormone is necessary to obtain adrenal atrophy in female than in male rats (Ingle 165) while the administration of estrogens will induce cortical hypertrophy (Selye, Collip and Thompson 166). Bourne and Zuckerman (50) have show that this will occur if only small doses of estrogens are injected, larger doses producing atrophy.

The role of the pituitary gland as an intermediary between

the two endocrine glands has been stressed by Golla and Reiss (168) who showed that in hypophysectomized rats with unilateral adrenalectomy no compensatory hypertrophy occurs. This contrasted with non-hypophysectomized animals whose adrenal weight increase was of the order of 60% and when estrone was injected, of 250%.

Schumaker and Firor (169) found a decreased gonad stimulating power in the pituitaries of adrenalectomized rats. A number of investigators concentrated their interest on the action of the adrenals on the estrous cycle.

Wyman (47) found that after bilateral adrenalectomy the diestrous phase will persist though the ovaries remained intact.

Moon (48) injected adrenocorticotropic hormone in spayed 22-day-old female rats and the vagina opened prematurely, earlier than the uninjected controls. Similar results were obtained by Corey and Britton (207).

The converse experiment was performed by Wade and Haselwood (49) who delayed the opening of the vagina in female rats with both adrenals and ovaries removed. No delay occurred in ovariectomized rats with adrenals conserved.

A very thorough study in this respect is that of Bourne and Zuckerman (50, 167) who showed that in ovariectomized female rats injected with a daily threshold dose of estrone the adrenals varied cyclically with a maximum increase at estrus. In adrenalectomized rats more estrone was necessary to obtain vaginal cornification and no characteristic cyclic variations

were produced though the pituitary gland was not removed.

Thus, the action of the adrenal glands on the estrous cycle being established on a triple morphological, physiological and chemical basis it seemed interesting to see what would be the behavior of these glands in regard to the known inactivating role of the liver.

Adult female rats of two strains (Sherman and Long-Evans) were segregated in groups of 5-10 animals and their estrous cycle followed daily. (Tables II & III)

1st group: normal controls;
2nd group: ovariectomized;
3rd group; ovariectomized-adrenalectomized;
4th group: ovariectomized-partially hepatectomized;
5th group: partially hepatectomized in "constant" estrus.

Each animal received sub-cutaneously 2 mg. of adrenocorticotropic hormone daily for five days.

Results The ovariectomized and ovariectomized-adrenalectomized rats (groups 2 and 3) deprived of their effector organs did not react and remained in anestrus. The opposite result was obtained in groups 4 and 5. The ovariectomized-partially hepatectomized rats had the leucocytes of the vaginal smear replaced by epithelial and cornified cells. The partially hepatectomized in "constant ~~cornified~~-estrus" became fully cornified. An intermediate position between groups 2-3 and 4-5 was maintained by the normal rats, they being unaffected by the injected adrenotropic hormone while in diestrus and

exhibiting prolonged cornification (3-4 days) when in estrus.

It is obvious from this experiment that the adrenals release estrogenic substances when stimulated by adrenocorticotropic substances. Also these substances apparently are like ovarian and exogenous estrogens in that they seem to be inactivated by an intact liver. However, in order to establish more definitely the similarity of behavior of the estrogen produced by the adrenals with the estrogens released by other producing sources (ovary, urine) the following experiment was devised.

Eight adult female rats of the Long-Evans strain were subjected to autoplastic grafting of both adrenals into the spleen. They were given an adequate salt solution to drink for the six weeks necessary for the grafts to become established (Wyman and tum Suden) personal communication. In the vaginal smears taken irregularly for the first five weeks, leucocytes were present with occasional epithelial or cornified cells. Starting in the 5 or 6th week tap water was substituted for the salt drinking solution and vaginal smears were taken daily for the next six weeks. No change in the normal cyclic aspect of the vaginal smear could be detected, implying that the presumed adrenal estrogens were not affected by their passage through the liver. In order to test the physiological activity of other adrenal cortical hormones several experiments were performed.

1) Resistance to cold. Five grafted rats were kept for an hour in the refrigerator at 5 C. No changes in the rectal temperature was noticed as compared to normal controls.
2) Blood sugar and liver glycogen determinations in five 24 hour fasted animals were between the limits of normality.
3) Life maintenance. Five animals were kept alive for more than three months and then killed. Three others lived for more than five months. They behaved like normal animals and maintained their initial weight.
4) Autopsy. The aspect of the grafted adrenals was normal and no adhesions were noticed. A microscopic examination did not reveal aberrant cortical tissue.

In conclusion, it would appear that the adrenal cortical hormones are not inactivated by the liver.

Discussion. Two preliminary points apparently without direct bearing on our problem have to be considered.

1) The adrenocorticotropic hormone used was supposed to contain no other pituitary substance except a small amount of lactogenic hormone. However, because of their protein nature and of the difficulties involved in their extraction it is impossible to ascertain that no gonadotropic or other hormones were contained in the fraction used. The response of group 2 seems to rule out this possibility at least partly.

2) The condition called "constant" estrus results in most of our partially hepatectomized rats.(70%) Besides its spontaneous occurrence (Long and Evans 21) it was obtained under the most various experimental conditions such as the grafting of the ovaries into the tail (Everett 173) by grafting testes from litter mates, or by putting rat females in parabiosis with castrated males (Pfeiffer 174). According to Witschi and Pfeiffer (175) the link between such different procedures is provided by the fact that in all cases the pituitary gland will release only FSH the production of LH being suppressed.

It should be noted that our animals were usually in "constant" pre-estrus with predominant epithelial cells.

These two orders of facts are striking examples of the intricate mechanism of the hormonal regulations inside the body and of the difficulties involved in their experimental approach and theoretical explanation. For the purpose of our discussion the "liver" aspect and the adrenal aspect of the problem will be considered separately.

That the liver plays a part in the metabolism of the endogenous adrenal estrogens is shown by the following fact. The vagina of the ovariectomized rats is not affected by adrenocorticotropic stimulation. It may be supposed that adrenal estrogens thus released are not quantitatively sufficient to overcome the inactivating power of the liver. On the

contrary, if the liver is deficient as a result of partial hepatectomy cornified cells appear in the vaginal smear as in the case of the ovariectomized-hepatectomized group. In the group of animals in "constant" estrus the amount of estrogens released by both ovaries and adrenals was insufficient to produce full cornification. The supplementary amount of estrogens was provided by adrenocorticotropic stimulation and it may be concluded that the adrenal and ovarian estrogens have an additive effect. The same conclusion was reached by Bourne and Zuckerman (50,167) according to whom the adrenals furnish the necessary amount of estrogens above the threshold dose to cause full estrus. The chemical isolation of estrone from ox adrenals (Beall 161) brings further support to this view.

In Moon's experiment (48) the integrity of the liver did not prevent the early opening of the vagina of rats injected with adrenocorticotropic hormone. There is no available indication that the liver of the immature rat would be less potent than the adult's in inactivating estrogens. It is possible that the estrogenic threshold for the opening of the vagina is lower than that producing cornification. In fact two of the five animals used in his experiment showed only leucocytes in the vaginal smear. The existence of threshold levels peculiar to each organ in respect to hormone activity is a generally accepted fact.

In conclusion our experiments on the adrenal estrogens fit well into the conception of the inactivation of estrogens by the liver.

However, when the above data are confronted with those resulting from the spleen-grafted adrenals experiment our conclusion cannot be accepted without caution. If the acrenals' participation in the regulation of the estrous cycle is through their estrogen secretion only and if this (or these) estrogen is similar to those produced by the ovaries or to their exogenuous equivalents, then it should be inactivated by the liver. Consequently, the estrous cycle of the adrenal grafted animals should under all experimental conditions be the same as in adrenalectomized animals. According to Wyman (47) confirmed by Grollman (170) and by Corey and Britton (207,208) bilateral adrenalectomy impedes the normal estrous cycle but surprisingly enough such was not the case in our animals. Thus other possibilities have to be considered.

One of them is that the adrenal glands act upon the estrous cycle through other hormones than the estrogenic. The relationship between estrogens and the water content of the organs affected by them is well established in the rat (Astwood 171 Zuckerman 209), dog (Thorn and Harrop 172), and primates (Gutkelch and Zuckerman 209), (Aykroyd and Zuckerman 210). It is plausible to assume that the adrenals interfere with the estrous cycle inasmuch as they regulate water

metabolism. This assertion is contradicted by the fact that the normal oestrous cycle in adrenalectomized rats is not reestablished by supplying adequate amounts of desoxycorticosterone. In our own experiment the substitution of top water for salt solution was not followed by any sign of adrenal insufficiency. Also the probability of any desoxycorticosterone-like product acting in the rat as an estrogen is unlikely. (Salmon 44)

Another possibility is that the estrogens produced by the transplanted adrenals is of a different chemical nature than that produced by the ovaries and because of this property more resistant to inactivation. If this is true, then, according to our experiment resistance to liver inactivation would be a general property of all adrenal steroids, estrogens or others. This is in agreement with previous work of Eversole, Edelman and Gaunt (211).

The same investigators found that pellets of desoxycorticosterone in the spleen implanted are only partly inactivated by the liver. By using the same technique Burrill and Greene (213) obtained the opposite result though Mark (214) is very definite in his claim that desoxycorticosterone is not inactivated by the liver. Such a discrepancy between the effects observed with exogenous and endogenous hormones supposedly identical is not without analogy in the physiology of the adrenals. Burrill and Greene (215, 216) found that the andro-

genic substances produced by the adrenals of immature castrated rats are not inactivated by the liver while pellets of testosterone and methyl testosterone are inactivated. In this respect it may also be assumed, only as a working hypothesis that the adrenal releases some precursor that is converted to an active estrogen after passing through the liver. This converting role of the liver will be discussed in another chapter.

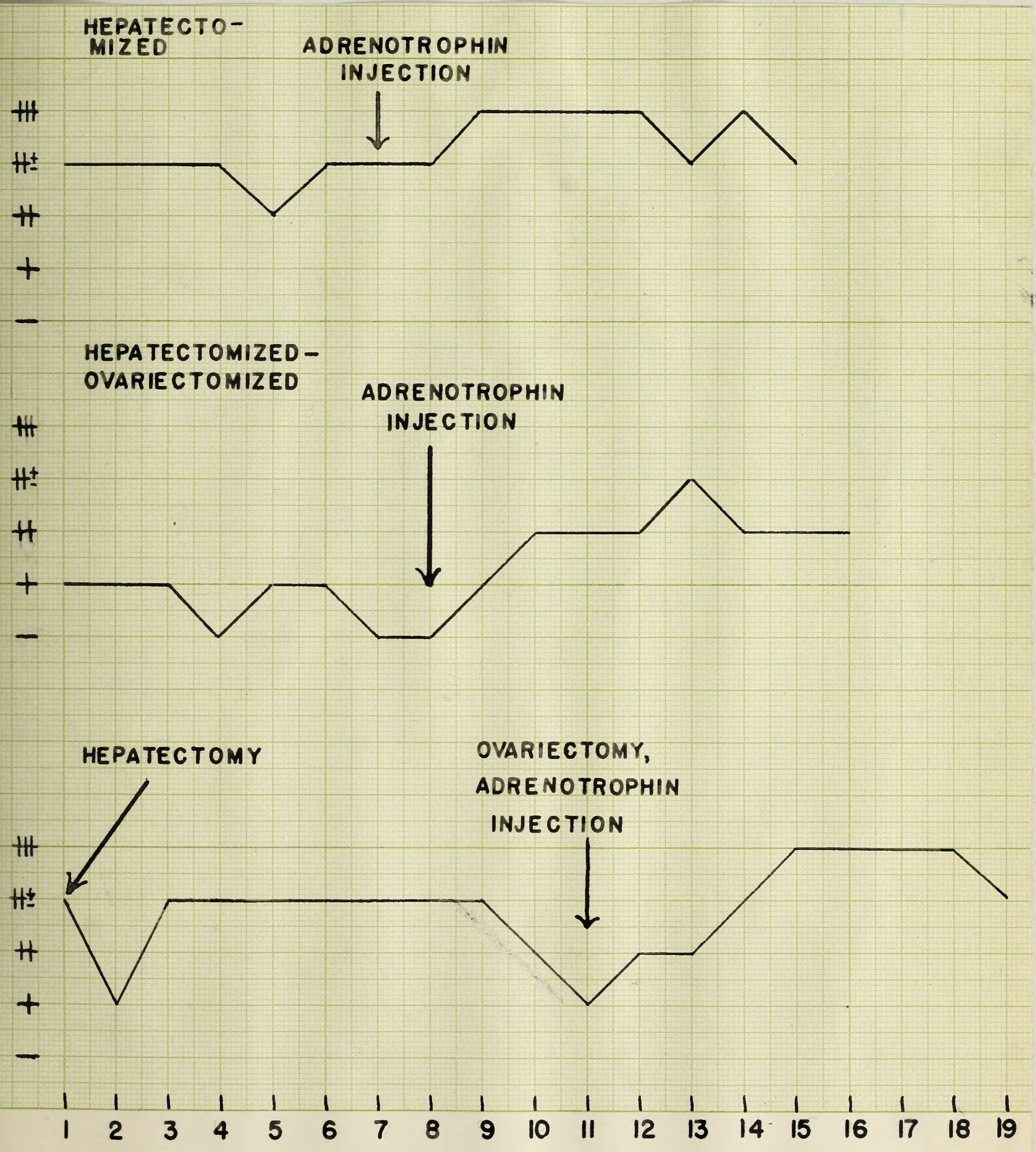
A last possibility is derived from the incompleteness of our investigation. No experiment was performed on hypophysectomized animals. It may be thought that in the cases where adrenocorticotrophic hormone was administered the injection stimulated the pituitary gland which in turn overstimulated the ovaries to release more estrogens. Such an assumption is, however, inadequate in the case of the injected ovariectomized rat (62) where no effector organ was present. It would be even more difficult to see what the stimulating mechanism would be in the case of the spleen grafted animals since Bourne and Zuckerman (50, 167) found that the cyclic changes of the adrenals are independent of the hypophysis.

It is not possible as yet to conciliate these contradictory facts. As aften occurs in experimental biology further development is necessary to show that such contradiction is only apparent and further investigation results in a new synthesis of the scattered facts.

Table II Effect of adrenocorticotropic hormone on the vaginal smear.

Number of Group	Type of Animal	Vaginal Smear	
		Before administration of 2 mg. adrenocorticotropic hormone.	After administration of 2 mg. adrenocorticotropic hormone.
1	Normal	Normal cycle.	1/Diestrus was affected. 2/ Estrus prolonged beyond normal.
2	Ovariectomized	Anestrus	Anestrus
3	Ovariectomized-adrenalectomized	Anestrus	Anestrus
4	Ovariectomized-partially hepatectomized.	Diestrus occaionally a few epithelial cells.	Pre-estrus-estrus (Epithelial and cornified cells)
5	Partially hepatectomized.	1/Constant estrus. Epithelial cells predominant. Few leucocytes and few cornified cells.	Full estrus (Cornified cells only).

Table III. Variations in the vaginal smear in partially hepatectomized rats.



Conclusions

1. The adrenals produce estrogenic substances which are most easily detected in partially hepatectomized female rats injected with adrenocorticotrophic hormone.
2. In such animals, the operative procedure induces a condition of "constant estrus" and the injection of hormone converts the vaginal epithelial cells to fully cornified cells.
3. Under similar conditions partially hepatectomized-ovariectomized rats have the vaginal smear changed from the leucocyte to the epithelial type.
4. Ovariectomized and ovariectomized-adrenalectomized rats are not affected by the hormone.
5. Estrogens produced in the adrenals are not inactivated by their passage through the liver.
6. The same conclusion holds for the adrenal produced hormones responsible for maintenance of life, resistance to cold, blood sugar level and liver glycogen.

The Fate of Injected Estrone in the Adult Male Rat

As seen in chapter IV, the bulk of evidence points to the liver as playing the major part in the inactivation of estrogens. Inactivation, however as a final phenomenon without the intermediary phases of which it is the end result. Also the problem of a possible quantitative relationship between the amount of liver tissue present and the rate of inactivation of estrogenic substances is ignored.

In order to investigate these points we decided to perform comparative experiments on normal and hepatectomized adult male rats. The choice of this animal was justified by the following considerations;

1. Previous reports on the catabolism of injected estrogens in the rat were contradictory (see chapter IV);
2. The rat is available in large numbers;
3. In order to obviate the variations due to the estrous cycle males were preferred to females;
4. Being the most used animal in the bioassay of human excreted estrogens it seemed interesting to see if such reliability could be explained in terms of patterns of catabolism similar in both species.

Experimental. Groups of 7-10 animals were used and segregated as follows:

1. Normal uninjected rats serving as controls;
2. Hepatectomized uninjected rats;
3. Normal rats injected with 50 micrograms of estrone per rat.
4. Hepatectomized rats injected with 50 micrograms of estrone

per animal.

The urines of both normal and hepatectomized uninjected rats were collected for 72 hours,The urines collected during the first 48 hours were kept separately from those collected in the succeeding 48 hours in the groups which received estrone.

Results.The data on urinary estrogenic activity are presented in Table IV.

1.Uninjected rats.The normal animals excreted in 72 hours detectable amounts of estrogenic substances practically all of which were contained in the "weak phenolic" fraction representing estradiol and estrone.The titer of the latter was higher than that of the former in a ratio of 3:1.

The hepatectomized animals while excreting only slightly higher amounts of estrone than the non-hepatectomized doubled the output of estradiol so that tha ratio estrone-estradiol was about 3:2.

In none of the two groups could estriol be considered as present.The very small amount of estriol indicated in the table were within the limit of error of the method employed.

2.Injected rats.In the non-hepatectomized animals the urinary recoveries of both estradiol and estrone fractions were greatly increased as compared to normal rats.During the first 48 hours the estradiol urinary output increased some 6 times and estrone some 4 times.During the second 48 hours the amount decreased in both fractions but estradiol still amounted to about twice and estrone to about 3 times that of normal rats.

A new fact of outstanding interest was the appearance of

estriol in the urine of the injected animals.The amount was appreciable since during the first 48 hours estriol represented 60% of all estrogens excreted and during the second 48 hours still one third of that amount.

The increase of all estrogenic fractions was most impressive in the hepatectomized injected rats,During the first 48 hours following injection the urinary increase of estradiol was twelve fold, of estrone eighteen fold while estriol represented 30% of the excreted estrogens.During the second 48 hours although the output decreased in all fractions,estradiol still measured six times that of the controls,estrone nine times and estriol 30% of the total estrogens.Tha calculated recoveries showed that the total output during the first 48 hours represented 13.6% of the injected estrone in the non-operated animals as against 44.8% inthe hepatectomized.During the second 48 hours the percentage was 5.1 in the non-operated and 20.7 in the pperated.Concerning the calculated recoveries of the injected estrone as expressed by the various metabolites(Table V)two facts are obvious :

1. that the proportion of estradiol remained constant for each collection period and from either type of animal(estradiol-estrone = 1.3);

2.that the proportion of estrone and estriol varies considerably when passing from the non-operated to the hepatectomized group. During the first 48 hours after injection the ratio of recovered estrone to estriol is approximately 1:22 in the non-operatedrats whereas it is approximately 1:08 in the hepatectomized rats; during the second 48 hours the corresponding ratios are 1:14and 1:06.

The importance of the liver in the conjugation of estrogens as found in other animal species and mainly in human beings suggested another experiment in order to see if the same phenomenon of conjugation also occurs in the rat.

Another group of 10 normal adult male rats was injected with 50 mg.of estrone each.The urine collected for 96 hours was divided into two equal aliquots:the first was hydrolized,the second extracted directly.In each case the total estrogens were bioassayed.The significant facts were:

1.that the recovered amount represented 12% of the injected estrone as compared to the 18.5% recovered in the previous experiment;

2.no difference was found between the hydrolized and the nonhydrolized fractions each one equaling 82.5r.u.(expressed as estrone).These data imply that all of the estrogens excreted into the urine are in the "free"state and not in the conjugated form.

Discussion.Before starting any further discussion a preliminary statement is necessary.We used the names"estradiol"for"weaknonketonic","estrone"for"weak ketonic"and"estriol"for"strong phenolic"fractions. This terminology was used only as a matter of convenience and based on the fact that these fractions behave during fractionation and bioassay the same way as crystalline estradiol,estrone and estriol. However,no strict chemical isolation has been undertaken because such a procedure would be impossible to apply to the small amounts of estrogens available to us.For biochemical procedures amounts of no less than 10.000r.u.

are necessary.

Also in chemical procedures a significant proportion is always lost and the deficit is higher than the 200 r.u.available in our experiments.As emphasized by Thayer,MacCorquodale and Doisy(178),the advantage of estrogen fractionation and partition methods resides in the fact that they can detect small amounts of estrogens unavailable to any other technique.

Also less material is lost in bioassay.In other words in this respect the animal is more sensitive than the chemical reaction.As a conclusion it can be stated that the substances extracted from rat urine in our experiments can be labeled at least as "estradiol-like","estrone-like",and "estriol-like" estrogens.

It was convenient for our discussion to separate the endogenous from the exogenous estrogens.

The endogenous estrogens were excreted in very small amounts thus our data confirmed the previous findings of D'Amour,Funk and Glendening(107),Dorfman(108)and Beerstecher(106).Dorfman is the only one to give data on the quantity excreted and his finding of 1 γg.of urinary estrogenic substances per rat per 48hours is only slightly above the 0.8$\gamma$of our experiment.

However,none of the previous investigators has attempted to fractionate the material in order to get more precise data about the nature of the excreted estrogens.In our experiments thenon-operated controls eliminated in the urine both estradiol and estrone the latter three times as the former. This ratio did not

obtain in hepatectomized controls where the output of estrone was only slightly increased (from 0.58 to 0.62)while that of estradiol was doubled(from0.19 to o.37)so that the ratio estrone: estradiol was about 3:2.This contrast between the constancy in both groups of animals and increase of estradiol in the hepatectomized group was the most striking feature and several explanations could be advanced.

Estradiol is the primary estrogen produced by the adult male rat and estrone is a product of its inactivation which in the case of estradiol occurs as a more rapid rate.The extent of the inactivation cannot be entirely ascertained but it is obvious that hepatectomy inhibits it.If all estrone is a product of a conversion from estradiol then there is a limit to such conversion beyond which no more estrone will be produced.This limit is indicated by the constancy of the estrone level in both normal and hepatectomized rats and the 25% to 30% of liver tissue left in the latter group will suffice to account for it.However,such an assumption is controverted by the fact that both estrone and estradiol have been isolated in various species from organs which, pending further information, are not sites of conversion.Also from some organs estrone alone has been isolated to the exclusion of estradiol.These facts will be considered in the following paragraph but it can be stated now that estrone is a primary estrogenic product.

No information is available from our experiments concerning the site of production of endogenous estrogens in the adult male

rat. Deductions can be made only by analogy with other species. Zondek(4) has furnished experimental evidence that the testis of the stallion is an estrogen manufacturing organ and Beall(43) isolated both estradiol and estrone from the testis of these species. Beall(161) also isolated estrone from ox adrenals but no estradiol could be obtained.

As a basis for further investigation it may be assumed that the same organs are producing the estrogen formed in the adult male rat. By all means hepatectomy by increasing the urinary output proves to be an excellent physiological technique for estrogen studies. Its usefulness is even more stressed by experiments with exogenous estrogens.

The urinary recoveries of the injected exogenous estrogens is larger in our experiment than in those of any previous investigators. With our 12-18% recovery we may contrast the recoveries of 0.6% obtained by Zondek(14,131),2-3% obtained by Dingemanse and Laqueur(132),and 0.5% obtained by Emmens(130). Such discrepancy can be explained in terms of differences in experimental procedures.Zondek investigated individual animals and the urinary estrogen output was too small to be usable. The same is true for the experiments of Dingemanse and Laqueur who used only two animals. Emmens injected his rats intraperitoneally thus,obliging all estrogen through the liver where it was inactivated.

Another reason for the discrepancy is to be found in the poor

techniques of hydrolysis and extraction available at the time when most of the experiments were performed. A good example is furnished by the increased recoveries of estrogens from human urines since more reliable techniques have become available. Dorfman who used them in his experiments on rats obtained a recovery of 10% which is close to ours. As it always occurs in the development of scientific investigation the progress accomplished is largely a function of the improvement of techniques. Doisy(seeThayer) emphasized the importance of such improvements for the recovery of small amounts of estrogens. However,the fact should be stressed that this aspect of our discussion is of a quantitative nature and that basically the phenomenon of inactivation remains unaltered and it amounts to over 80% of the injected estrone.

The analysis of the data on the excretion of the various estrogenic fractions reveals the notable fact that the injection of estrone induces an increase in the excretion not only of estrone but of estradiol as well. From what we know about the amount of estradiol normally excreted by the adult male rat the endogenous sources cannot account for it.

The only possible conclusion is that the difference in estradiol output between controls and estrone injected rats is due to estrone conversion. The same conclusion was reached by Heller(145,146) who incubated rat liver slices with estrone. Supplementary evidence in favor of a conversion mechanism is found in the fact that an entirely new metabolic product appeared in the urine which has chemical and biological properties similar to

estriol. - Estriol which is the main catabolic product is not a natural metabolite in the rat at least within the accuracy of the techniques actually available. The appearance of estriol is interesting because the number of species capable of converting exogenous estrone to estriol is limited. Dingemanse and Tyslowitz (123) recovered non Ketonic estrogens from the urine of estrone injected dogs but no fractionation was performed in order to ascertain the presence of estriol. Longwell and McKee indicate however, that much conversion is possible in dogs. Only recently Pearlman and Pearlman (179) claimed that conversion of estrone to estriol is possible in the rabbit. However the human species is the only one in which estriol occurs normally and as a conversion product of estrone. This type of conversion is corroboated by the results of the experiments to be described in the following chapter when definite conclusions as to the patterns of conversion in rats and human beings will be drawn.

As in the case of the endogenous estrogens the technique of hepatectomy furnishes strong indications that the liver is one of the most important factors (if not the most important) in the conversion from one estrogen to another. But before discussing this point a preliminary consideration of the experimental conditions is necessary. All our experiments on hepatectomized rats were performed while the livers were in full regeneration. Higgins and Anderson(24), Brues, Drury and Brues(180) showed that the regeneration of the partially extirpated liver is complete in 2 to 3 weeks but also (Higgins and Anderson 24) that anatomical restoration is not accompanied by functional restitution. The latter occurs at a slower

rate and our data on estrogen recovery confirm such a conclusion.

The total recovery in the partially hepatectomized rats amounts to two thirds of the injected estrone as compared to less than one fifth in non-operated animals. A definite relationship exists between the amount of liver tissue actually active and that of the inactivated estrogen since only 35% of it cannot be accounted for.

The degree of interconvertibility as resulting from the analysis of the three fractions furnishes even more significant data. Though all three fractions are increased the conversion of estrone to the more potent estrogen estradiol and to the less potent estriol is subjected to variations which differ from those found in the normal injected rats. More estrone is recovered unchanged and the estriol fraction is more affected by this reduction of conversion than the estriol fraction. It is clear that the body has lost part of its power of inactivation and conversion from estrone to estriol.

The ratio of estradiol to estrone is practically unaltered by hepatectomy in the injected rats. This would imply that the degree of conversion from estrone to estradiol reaches an equilibrium beyond which no more estrone is converted and the actually active liver tissue suffices to assure it. Another possibility may be that in the over-all bodily activities the liver plays a minor role and some other organ is mainly involved.

There remains the problem of the fate of the substances rendered inactive. Several explanations have been proposed concerning their behavior but none is satisfactory.

Excretion in the conjugated form does not occur in the rat. This is most surprising since most of the species do conjugate, mainly when estriol is the end product.For instance in the human species conjugation occurs without exception and the consequent extraction method became a common routine. Our experiment on the equal recovery of hydrolized and nonhydrolized urine samples excludes this form of inactivation in the rat. Similar conclusions were reached by Zondek(14), Dingemanse and Laqueur(132) and Heller. However it is generally accepted that hydrolysis methods are not perfect and perhaps this is even truer in rat urine.

Nor are the unaccounted estrogens to be found in the feces. Dingemanse and Laqueur(132) in experiments on the rat could account for only 3% of the injected estrone. In a preliminary experiment we obtained under similar conditions a recovery of the same order of magnitude.

The possible role of enzyme systems has received consideration from Zondek and Sklow ( ) and from Westerfeld (154) who inactivated estrogens with enzymes extracted from plants, but Graubard and Pincus (155) were unable to obtain phenolase from animal livers.

Cantarow and his co-workers(148) as a result of their experiments on dogs suggested that thebile could be a site of estrogen storage and excretion. The attempts of Zondek (14) and Dingemanse and Laqueur(132) to extract the injected estrone from the whole animal do not support such a view, at least in the rat.

A new hypthosis was advanced by Cantarow and his colleagues according to which the liver stores the injected estrogen in an inactive form and reexcretes it after "reactivation". Two criticisms

can be formulated against such views. The inactive form was not identified and the "reactivated" estrogen was not fractionated thus leaving open the probability that the injected estrone could have been converted to the more active estradiol. Our experiment on mormal and hepatectomized male rats showed that such conversion does occur but throws no light upon the nature of the inactive substances.

Table IV.

TABLE IV THE URINARY ESTROGEN OF NORMAL AND HEPATECTOMIZED MALE RATS BEFORE AND AFTER THE INJECTION OF 50 MICROGRAMS OF ESTRONE PER RAT

Type of Animal	No. of Animals in Group	Injected or Uninjected	Period of Urine Collection	Urinary estrogen in micrograms per rat per forty-eight hours: Non-ketonic Weak Phenolic (as α-Estradiol)	Ketonic Weak Phenolic (as Estrone)	Strong Phenolic (as Estriol)
Normal	10	Uninjected	72 hrs.	0.19	0.58	<0.03
Hepatectomized	8	Uninjected	72 hrs.	0.37	0.62	<0.04
Normal	10	Injected	1st 48 hrs. after injection	1.17	2.40	4.00
Hepatectomized	7	Injected	1st 48 hrs. after injection	4.03	11.40	7.94
Normal	10	Injected	2nd 48 hrs. after injection	0.50	1.54	1.29
Hepatectomized	7	Injected	2nd 48 hrs. after injection	1.77	5.10	3.47

Table V.

TABLE V THE CALCULATED RECOVERIES OF INJECTED ESTRONE AS THE VARIOUS URINARY METABOLITES (SEE TEXT)

Type of Animal	Collection Period	Estrogen recovered per rat: Estradiol		Estrone		Estriol		Totals	
		μ.gm.	%	μ.gm.	%	μ.gm.	%	μ.gm.	%
Normal	1st 48 hrs. after injection	0.97	1.9	1.82	3.6	4.00	8.0	6.79	13.6
Hepatectomized	1st 48 hrs. after injection	3.66	7.3	10.79	21.6	7.94	15.9	22.39	44.8
Normal	2d 48 hrs. after injection	0.30	0.6	0.96	1.9	1.29	2.6	2.55	5.1
Hepatectomized	2d 48 hrs. after injection	1.77	3.5	5.10	10.2	3.47	6.9	10.34	20.7

Conclusions

1. The adult male rat normally excretes estradiol and estrone in small but detectable amounts in the urine.

2. Partial hepatectomy increases the output of estradiol and slightly that of estrone.

3. Injection of estrone increases the output of both fractions and induces the appearance of estriol in the urine.

4. Two roles are assigned to the liver:
   (a) inactivation of over 80% of the injected estrone.
   (b) conversion of estrone to estradiol and estriol.

5. The estrogens are excreted in the "free" form.

6. Partial hepatectomy increases the output of all fractions but their ratios are changed and less estrone is inactivated and converted.

7. There is a parallelism between the amount of liver present and the amount and rate of inactivation and conversion during a given time.

8. The suggestion is made that another organ besides the liver may play a part in these phenomena.

The Perfusion of Rat Organs with Estrogens in vitro.

The experiments described in the preceding chapter afford first rate results concerning interrelationships between the effector organism and the stimulating estrogens as illustrated by the phenomena of conversion and inactivation. However, they leave unanswered a number of problems without which a clearer understanding of this interrelationship is incomplete. These problems concern the possible different behaviour of the endogenous as compared to the exogenous estrogens. Also the exact details of the conversion of not only estrone but estradiol and estriol as well needs clarifying.

As a result of the experiments on hepatectomized rats it was hinted that although the liver has a major rôle other organs probably play a part in the over-all bodily activities and have a share in the processes involving the metabolism of estrogens.

In these experiments male rats were used but it would be unjustified to apply to female rats conclusions drawn from experiments on males since sex differences might interfere Moreover the common use of female rats for routine work in human clinics justifies experiments on this sex.

Finally by changing the experimental conditions it would perhaps be possible to gain a better insight into the

nature of the inactive end products. All these problems are intimately connected with each other and it seemed to us that in order to dissociate them other physiological techniques of a more analytical character would reveal more precise results than would the techniques involving the reactions of the whole body. The transition from the "body-level" to the "organ-level" could best be assured by organ perfusion experiments in vitro and this technique was adopted. Such a method of investigation has the advantage of permitting:

(1) the use of an isolated organ,

(2) the use of an estrogen of a definite type,

(3) timing in the succession of the various stages,

(4) the exclusion of all other factors.

Experimental.

The technique of organ perfusion has been described (chapter III). In all cases organs of adult female rats of the Sherman strain were used. In order to keep all conditions uniform the selected animals were in the pro-estrus stage as shown by the vaginal smear taken during the few minutes preceding the operation.

Preliminary experiments were performed on the perfusing media. The use of defibrinated rat blood was justified as a result of previous work by Zondek (14) who showed that its power of inactivation was nil. For all

practical purposes, however rabbit serum had many advantages and it was decided to use it. Submitted to tests in order to detect any possible estrogenic contant it was found that 18 cc.of serum were devoid of any estrogenic activity(less than 1 r.u. in 18 c.c. of serum).Incubation for 3 and 17 hours of rabbit serum with 300 r.u.of α-estradiol permitted a complete recovery of the added estrogen thus eliminating any suspicion of inactivation. This is in agreement with Zondek's conclusions on rat blood(14).As far as Locke's solution is concerned it is ~~of~~ common knowledge that it is not a favorable medium for either inactivation or potentiation of estrogens.

Results.(Table VI).

Liver. Perfusion with 300 r.u. of α-estradiol for three hours resulted in a loss of 85% of the active substance (Exp.6).When larger amounts of α-estradiol were used for the same duration the loss of activity decreased to 69 % of the added 3200 r.u.(Exp.2 and 3) and 71% of the 4080 r.u. of α-estradiol(Exp.4).With increased perfusion time (6 hours) there was a further loss of activity from the perfusate amounting to 82% whem 3200 r.u.were used(Exp.3) and 92% when 1664 r.u.were used(Exp.5).Perfusion of the liver for 5 hours with 208 r.u. which represents the smallest amount of α-estradiol used,left in the perfusate only 15 r.u.(Exp.7).This level is the same as that found

after perfusing a fresh liver for three hours without adding any estrogen(Exp.8).

The loss of activity was not due exclusively to inactivation,but also to conversion of α-estradiol to the less potent estrogens,estrone and estriol,which in turn were inactivated.The ratio of conversion α-estradiol⟶estrone ⟶estriol was about 2:2:1.

Twice(Exp.4 and 5)aliquots were hydrolyzed in order to detect any possible conjugation of estrogens but none was found.

Perfusions with estrone resulted in a loss of activity similar to that found for α-estradiol but the rate of inactivation is slower since only 17% was inactivated after 3 hours(Exp.9 and 10),53% after 6 hours(Exp.10) and 78% after 10 hours(Exp.11).Also the products of conversion represent values slightly above those resulting from the conversion of α-estradiol. The latter is the most permanent beneficiary and its values as expressed in r.u. are above those of estrone independently of the duration of the perfusion.

Perfusion with estriol (Exp.12) showed an inactivation of 30% in 3 hours and no conversion to other estrogens.As in all experiments in which conjugation was suspected here too hydrolysis was performed but no increase in estrogenic potency found.

Kidney.The perfusion of these organs with α-estradiol led to a loss of activity similar to that produced by the liver

but the rate of inactivation wasless rapid. It averaged only 53% after 3 hours(Exp.13 and 14),65% after 6 hours (Exp.14 and 15) and 97 % after 10 hours(Exp.15). As in the liver here too conversion occurs but ~~xx~~ although the production of estrone is indisputable that of estriol has to be accepted more cautiously.

Heart. The perfusion experiments with liver and kidney did not exclude the possibility of other organs having the property of converting and inactivating estrogens. The heart was a tempting organ on which to test it. A 3 hour perfusion with 300 r.u. of α-estradiol permitted an almost total recovery,the 30 r.u. lost(Exp.1)being within the limits of error of the extraction procedure. The recovered estrogen was in the estradiol fraction consequently no conversion occured.

Liver content of estrogenic substances. In order to exclude the possibility that the estrogens disappearing from the perfusate are stored in the liver the estrogenic content of this organ under normal and experimental conditions was investigated~~x~~(TableVII).The technique used is described in chapterIII .Two fresh normal livers yielded an amount of 28.6 r.u.,i.e.,2.6 r.u. per gram of tissue. The same type of liver perfused for 3 hours without any added estrogen showed practically no difference with the preceding result the yield being of 2.13 r.u. per gram of tissue.A 3 hour

perfusion(Exp.9) with estrone ~~permitted~~ a recovery from the liver tissue of the three fractions, estradiol assuming more than estrone and estriol.

Discussion. These experiments confirm the indisputable fact that the rat liver is a major site of estrogen inactivation. In chapter IV a survey of the findings of other investigators was made and their repetition is not necessary.

The rôle of the kidney in inactivation is less known and previous experimental work is limited to that of Fee, Marrian and Parkes (135) who after perfusing a dog's heart-lung-kidney system found that only 1% of the added estrogen was excreted through the kidney. However their interpretation of the facts was erroneous since they attributed the inactivation to an oxidative process occuring in the lungs thus discarding entirely the kidney. Heller (145) by incubating estradiol and estrone with rat and rabbit kidney found a partial inactivation. Our data correct the interpretation of Fee, Marrian and Parkes (135) and also bring evidence that a complete inactivation of α-estradiol by the kidney is possible if the time factor is not neglected. The most striking result of our experiments as compared to those of other investigators is that none of them has detected any conversion from one estrogen to another and this needs explanation. Most of the previous experiments were done exclu-

sively on a qualitative basis taking the vaginal smear as an indicator. Such was the case in the experiments of Golden and Severinghaus (133),Biskind and Mark (140),Biskind (141),Segaloff and Nelson (142),Talbot (137),Pincus and Martin (138),Twombly (147).

Other investigators although having both qualitative and quantitative approaches to the problem also missed the conversion. Israel,Meranze and Johnston (144) who used methods similar to ours(perfusion of dog liver with 1000 r.u.of estrone and bioassay in rats) found that inactivation was a matter of minutes. Also Haller by incubating liver slices with estradiol and estrone ignores it. In our experiments all factors which occasionally would impede the detection and analysis of conversion were considered. The liver is an organ having such a high power of inactivation that it was necessary to flood it with estrogens beyond its working capacity in order to slow down the mechanism of inactivation.

Israel and his co-workers used only 1000 r.u. of estrone for a dog liver. If we assume that a dog liver averages 350 gms. than this means that the ratio was of almost 3 r.u. per gram of tissue as compared to the 375 r.u. of estrone of our experiments. The same objection can be formulated against Heller who used the minute amount of 0.3 γ estradiol for 250 gms. of liver tissue. It can be argued against our proce-

dure that an organ saturated with such a large amount of estrogen is in far from physiological conditions. This argument loses its value when it is remembered that the perfusion experiments check well with those performed on normal and hepatectomized rats where only 50 r.u. per rat were used.

The time factor was considered by Israel(144) who took an aliquot of the perfusate every 15 minutes but in concentrating his interest on the rate of inactivation he made no attempt to determine if the obtained estrogen was different from the initial one. Heller used in all his experiments the standard time of 1 hour. Under such circumstances the rate and direction of conversion could not be investigated. Briefly the steps of conversion could be adequately followed only by coordinating such different factors as nature and amount of the estrogen initially added, timing, rate of production and identification of the final products.

xxxxxxx Our data xxxxxxxxxxx show that the liver is a powerful converting organ of estrogens. Conversion is overshadowed by inactivation which occurs at a very high speed but reaches a limit as shown by the fact that over 2000 r.u. of α-estradiol were inactivated during the first 3 hours while only slightly over 400 r.u. were inactivated from the 3rd to the 6th hour when an equilibrum between the various

fractions seems to be reached. This is apparently not the case for estrone but when it is considered that estrone is partly converted to estradiol which is eight times as potent as estrone the difference between the two estrogens though real becomes less significant. The direction of conversion corroborates data obtained from the experiments on normal and hepatectomized rats i.e., estrone is converted to both estradiol and estriol. In addition it shows that estradiol is subjected to the same type of conversion as estrone while estriol is the more resistant to inactivation and is not convertible to any other estrogen. The greater resistance of estriol to inactivation and its lack of convertability has been known since the experiments of Thayer(cited by Doisy 78) in monkeys, Smith and Smith (15), and Schiller and Pincus(113) in humans.

A general scheme of the occuring phenomena in the rat can be drawn as follows:

$$\begin{tikzcd}
\text{estradiol} \arrow[rr, shift left] \arrow[dr] & & \text{estrone} \arrow[ll, shift left] \arrow[dl] \\
& \text{estriol} &
\end{tikzcd}$$

This scheme is entirely similar to that proposed for the metabolism of estrogens in the human species(ChapterIV). It is noteworthy that such distant species follow similar patterns of estrogen metabolism. This could account for the reliability of the rat as a test animal in the bioassay of estrogenic products of human origin. It would be interesting

to see how the mouse reacts in this respect.

The converting role of the kidney was indicated by Heller(145)for estrone but no definite proof is given about the end product obtained. According to our experiments the kidney inactivates and converts estrogens at a slower rate than the liver. Also the direction of conversion seems to differ for the two organs. The production of estrone from α-estradiol is obvious in both cases but differ markedely for estriol. The amounts recovered after estriol perfusion are not large enough to eliminate definitely any fractionation error. Therefore the conclusion is justified that inside the body there are differences in the activity of various organs participating in the same physiological processes.

Another point of interest is the different behaviour of the liver in respect to the endogenous as compared to the exogenous estrogens. Three hours suffice to inactivate 2000 r.u. of α-estradiol while the 15 r.u.of endogenous estrogen normally contained by the liver of a female rat in proestrus are not inactivated. The same amount was recovered from hashed rat liver(Table VII) while under similar conditions exogenous estrogens were rapidly inactivated(Zondek 14).The same resistance of endogenous estrogens to liver inactivation was found in the experiments with spleen grafted adrenals (Chapter V). The implication is that although endogenous estro-

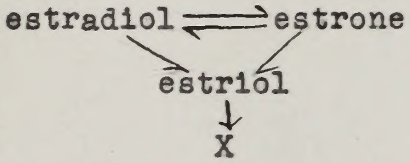
gens may be isolated from animal fluids by chemical procedures as estradiol ,estrone, and estriol under natural conditions either their chemical nature is different or some physiological mechanism protects them from rapid destruction. It is probable that the time is not far away when a new function will be ascribed to the liver. The work initiated by Emmens (181) suggests that synthetic compounds without estrogenic activity become estrogenic after being carried through the systemic circulation. About 20 such substances called by Emmens pro-estrogens have shown this property. Though he makes no mention of the site of conversion we have preliminary data indicating that at least one of them the 4:4'-Dehydroxy-diphenyl methane is estrogenic after passing through the liver. This may be an indication that the liver besides inactivation can potentiate estrogens. Conversion of estrone to the more potent estradiol is another proof.

As in the previous experiments (Chapter V)and in agreement with the work of Dingemanse and Laqueur(132)and of Heller (145)the perfusion experiments show no metabolite appearing in the conjugated form but"free".

No light is thrown upon the fate of the inactive products. The experiment (Exp.9 Table VI) devised to investigate this point was misconceived. The amount of estrogen used was too large and the perfusion time too short to allow any conclusion excepting that conversion was under way. The phase at which

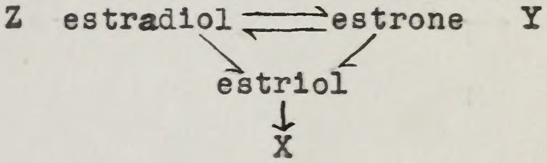
inactivation occurs is not known and anyone of the following possibilities awaits confirmation.

(1)The X substance derives from estriol:



or

(2)Each estrogen is subjected to a secondary inactivation :



As long as the chemical nature of these substances is unknown and no physiological techniques to elucidate it can be devised no choice is justified. It is also possible that experimentation at the "body"or"organ-level" could not account for such changes and investigations at the "cell-level" will be necessary as already suggested by work on enzymes.

Table VI The Recovery of Estrogenic Activity from

Exp.#	Organ perfused	Perfusion medium.	Estrogen in perfusate (R.U.)	Perfusion time (hours)
1	Heart	50cc.rabbit serum 75cc. Ringer-Locke	- estradiol 300	3
2	Liver	25cc.defibrinated blood plus 100cc.Ringer-Locke	- estradiol 3200	3
3	"	20cc.defibrinated blood plus 100cc.Ringer-Locke	- estradiol 3200	6
4	"	25cc.defibrin. blood plus 100cc.R.-Locke	- estrad. 4080	3
5	"	80cc. rabbit 15 Locke	- estrad. 1664	6
6	"	25cc.defibrin.bl.100Locke	- estrad.300	3
7	"	20cc.defibrin.bl.100Locke	- estrad.208	5
8	"	25cc.defibrin.bl.100Locke	0	3
9	"	75cc. rabbit serum 50 Locke	estrone 3000	3
10	"	75cc.rabbit s.50 Locke	" "	6
11	"	125cc.rabbit s.10 Locke	" "	10
12	"	75cc.rabbit s.50 Locke	estriol 2000	3
13	Kidney	75cc.rabbit s.50 Locke	- estrad4160	3
14	"	75cc.rabbit s.50 Locke	- estrad.4160	6
15	"	75cc.rabbit s.50 Locke	- estrad.4160	10

1 r.u. -estradiol = 0.125 micrograms

1 r.u. estrone = 1.0 "

1 r.u. estriol = 1.0 "

regular extraction

after acid hydrolisis

the perfusing medium in various perfusions of rat organs *in vitro.*

Activity recovered from perfusate (r.u.)				Remarks.
Total	N.K.(estradiol	K.(estrone)	Estriol	
270	270	4	0	
1000	400	400	200	
570	220	220	132	An aliquot taken at 3hrs assayed1000ru.
1200 1000	—	—	—	1/4of total plus 416ru. -estrad.assayed 700ru.1/4of total acid hydrol.plus416 -estr.assayed730ru.
134 128	—	—	—	
43	—	—	—	
15	—	—	—	
14	—	—	—	Liver weight 6.6gms
2520	936	798	663	
1410	663	400	200	Aliquot taken at 3 hrs.assayed2500r.u.
—	327	252	76	
1400	less than 10r.u.in benzene		1400	An aliquot hydrolized assayed 1400r.u.
1860	876	711	41	
1425	615	500	70	Aliquot at 3hrs= 2000 r.u.
124	50	28	12	Aliquot at 6 hrs.= 1500 r.u.

Conclusions.

(1) Perfusion xxxxxxxxxxx *in vitro* is a valuable technique for the study of (a) the role of various organs in the catabolism of estrogens, and (b) the dissociation of the various stages of conversion and inactivation;

(2) The amount of the added estrogen, its nature, perfusion time and identification of the products at successive phases are all important factors for a clearer understanding of both conversion and inactivation;

(3) Rabbit blood serum used as a perfusing medium has no inactivating or potentiating ability with respect to estrogens;

(4) The conclusions reached in the preceding chapter concerning the qualitative and quantitative effects of the liver on estrogen conversion and inactivation is stressed. Also a new role is ascribed to the liver that of potentiating estrogenic and pro-estrogenic substances;

(5) The kidney converts α-estradiol to estrone before inactivating it but the conversion to estriol is more disputable;

(6) The heart when perfused with α-estradiol induces no changes thus showing that the property of converting and inactivating estrogens is not a privilege of all organs;

(7) The direction of conversion follows the same pattern as that of the human species and it is suggested that for this reason the rat is the best test animal for the bioassay of human estrogens;

(8) The endogenous estrogens are not affected by the liver as shown by the constancy of the 15 r.u.contained in the liver of the female rat in pro-estrus.

(9) No evidence is obtained from these experiments concerning the fate of the inactive end-products.

Table VII The estrogen content of control and experimental rat livers.

Exp.	Treatment.	Estrogen content in r.u. per liver					Total r.u.
		Acetone extract				Liver Hydrolysate	
		Total	Estradiol	Estrone	Estriol		
—	Two fresh livers, no treatment*	9.9	—	—	—	18.7	28.6
8	3 hours perfusion, no estrogen†	‡	—	—	—	‡	14.1
9	3 hours perfusion, 3000 r.u. estrone	700	271	168	168	116	816

* Content per gram 2.68 r.u.

† " " " 2.13 r.u.

‡ The acetone and hydrolysate were combined.

VI. General Discussion.

The main object of our discussion concerns the role of various organs in the metabolism of estrogens.

The liver as an inactivating organ has been known since the pioneer work of Zondek(14),and repeatedly confirmed since. However,no attempt has been made to integrate it in a more general system of regulatory mechanisms of hormone metabolism. Since inactivation was the only known fact the process was represented as a quantitative one according to which all estrogens reaching the liver were destroyed while the amount recovered in the urine represented a fraction escaping inactivation by either rapid passage through the liver or direct passage into the systemic circulation. The finding of conversion products in body fluids did not reveal the precise location where this conversion occured. Early investigators (Pincus and Zahl 126) logically thought that the effector organs were the sites of conversion although all experiments on ovariectomized-hysterectomized mammals pleaded against such a view.(Chapter IV).

In fact the presence or absence of secondary sex organs proved to be immaterial. It is nonetheless surprising to find that organs like the liver and the kidney are mostly involved in such processes.

The role of the liver appears more complex and besides inactivation,two more properties can be ascribed to this organ: conversion and potentiation(perhaps synthesis)of estro-

gens and their precursors.

The activity of the liver seems to be cyclic as would be expected from an organ involved in endocrine processes. For instance the rate of inactivation is not the same throughout the perfusion experiments but varies in rate and intensity. Should inactivation occur without fluctuation then it would be difficult to account for the presence of small but still active amounts of estrogenic substances as the 15r.u. found in the liver of rat females during the follicular phase. The amount saved largely suffices to ensure the needs in estrogens during this phase of the cycle. It is not unreasonable to assume that the 15 r.u. found in three instances represents the approximate threshold for the liver and that converting-inactivating phenomena will occur above this level which in this way represents a threshold. Also it is noteworthy that perfusion with over 200 r.u. of α- estradiol leaves after 5 hours the same 15 r.u. Wether it represents a sequel of the added estrogen or wether the liver contained it already is difficult to assert.

The solution of this point would be important for the appreciation of the differential behaviour of the liver in respect to endogenous and exogenous estrogens. If endogenous estrogens are more resistant to inactivation as seems probable,then,the liver can discriminate between them and the exogenous estrogens. This is substantiated by the fact that

adrenal-produced estrogens also escape inactivation. However such escape is not thorough since the diestrus phase in both normal and ovariectomized non-hepatectomized rats is not affected by the presence of the adrenal glands. Here again we arrive at the idea of a threshold.

If the liver is a threshold organ then it can be included in the same category as all organs affected by estrogens. In fact most organs and many functions in the body(if not all) are affected by estrogens(Allen et.al., 182, Robson 184), and there is no reason a priori for the liver to be an exception.

The kidney is probably subjected to the same rules as the liver and the difference in rates of metabolism between the two organs leaves the basic facts unchanged. Together they constitute a powerful barrier which can account for the inactivation of over 4/5 of the injected estrogens.

These facts complete and complicate the problem of the regulatory mechanism of sex hormones. Of the factors involved several are known. The reciprocal activity of the pituitary-gonad system is classical since the work of Smith(185, 186). The quantitative relationship of estrogenic and luteinizing hormone has been thoroughly investigated by Hisaw(187, 188),Selye and Clarke(189),Courrier(190,191),Robson(193, 194,195),Zuckerman(205),Leonard et.al.,(201).

Less subjected to quantitative estimation is the receptivity of any effector tissue for the action of hormones. Its

importance has been stressed by Oesting and Webster(74), Dorfman et.al.,(192),Saunders and Cole(77),Dingemanse et. al.,(75),Hisaw(188)and Allen(196).

A fourth factor has to be added to the previous three: the organ factor as represented by the liver and kidney.It is not yet possible to integrate them all in one system similar to that of homeostasis for example. The Bernard and Cannon of the physiology of sex hormones are yet to come.

The unexpected fact is that the liver or kidney may play such a role. In the case of the liver for instance, behind the anatomical unity as represented by the hepatic cell lies a multiplicity of functions which no other organ assumes in the body. Its role in estrogen metabolism is contrary to the idea of specificity of structure and function in hormonal regulation as usually accepted. Such an attitude was justified by the discovery of adrenalin,insulin or thyroxin. However,increasing evidence is accumulating indicating that specificity is a matter of interpretation.

Specificity of estrogenic occurence in nature is untenable. Estrogenic substances have been found in the three natural kingdoms and among animals in a great number of species(Chapter IV).It is generally agreed that such estrogenic substances are a by-product of the general metabolism of the animal and have no function except perhaps a nutritive one (Lwoff 197).

Their estrogenic activity has been verified in mammals and it can be said that estrogens existed before estrus. This is not without analogy in nature: the corpus luteum has been described in reptiles (Rahm 198); L H in birds which have no corpus luteum(Gorbman 102); and prolactin in fishes, amphibians and reptiles(Leblond and Noble 199) which have no mammary glands. Such facts can be explained in terms of evolution only by assuming that estrogens acquired more specificity during phylogenesis as atructures and functions became more specialized.

Trends of hormone evolution were different and related species followed different patterns of estrogen metabolism(See example of primates, Chapter IV).

When ontogenesis is considered, the same lack of specificity, though more limited, is found.

The number of estrogen-producing organs is four and pleads against any exclusive specialization. As compared to the thyroid gland for instance the latter probably represents a more advanced step.

No organ produces estrogens exclusively but other hormones as well; testes produce estrogens(Zondek 4), Beall (43), ovaries produce androgens(Hill and Strong 200), and the adrenal glands produce both(Reichstein 202), Kendall (206). This is consistent with the existance of such substances as dehydro-androsterone and trans-androstenediol which have

both "male"and "female" properties(Butenandt 203,204).

Of the three properties,morphological,chemical and physiological considered specific from the classical viewpoint in endocrinology no single one survives the test of modern experimentation. This lack of narrow specificity of structure and function of estrogenic products can best be explained in terms of evolution. It is possible that in this respect the estrogens are on theirway of becoming more specific. Their metabolism which is now performed by the liver in conjunction with other organs and tissues such as the kidney,fibro-muscular tissue,and partly by the secondary sex organs may eventually be taken over by the latter.

VII. Bibliography.

(1) Allen, E. & Doisy, E.A. (1923) An ovarian hormone; Preliminary report on its localization, extraction & partial purification & action in test animals. J.A.M. A. 81:819.

(2) Stockard, C.R., & Papanicolaou, G.N., 1917. The existence of a typical oestrous cycle in the guinea-pig with a study of its histological & physiological changes. Am. J. Anat., 22:225.

(3) Aschheim, S, & Zondek, B. 1927. Hypophysensorderlaffenhormon und Ovarialhormon im Harn van Schwangeren. Klin. Welenschr. 6:1322.

(4) Zondek, B. 1934. Mass excretion of estrogenic hormone in the urine of the stallion. Nature, 133:209.

(5) Doisy, E.A., Veler, C.D., & Thayer, S.A. 1929. Folliculin from the urine of pregnant women. An. J. Physiol. 90:329.

(6) Butenandt, A. 1929. Uber "Progynon" eine krystallisiertes weibliches Sexualhormon. Naturwissenschaften, 17:878.

(7) Cohen, S.L. and Marrian, G.F. 1936. The isolation and identification of a combined form of estriol in human pregnancy urine. Boichem. J., 30:57.

(8) Girard, A. & Sandulesco, G. Sur une nouvelle serie de reactifs du groupe carbonyle, leur utilisation a l'extraction des substances cetonique et la caracterisation microchemiques des aldehydes et cetones. Helv. Chim. Acta 19:1095.

(9) Carrel, A. The culture of organs. 1938. P.B. Hoeber, New York.

(10) Wyman, L.C. & tum Suden, C. 1932. The growth of transplanted cortical tissue in the rat. Am. J. Physiol. 101:662.

(11) Id. 1937 Factors determining and limiting the growth of transplanted suprarenal cortical tissue. Endocrin. 21:523.

(12) Doisy, E.A. 1942. The estrogenic substances. Chap. XI p. 169 in Glandular physiology and therapy. Am. Med. Assoc. Chicago.

(13) Kober, S. 1931, Eins Kolorimetrische Bestimmureg des Brunsthormones. Biochem. Ztschr. 239:209.

(14) Zondek, B. 1934, Uber das Schiksal des Follikelhormones im Organismus. Scand. Arch. F. Physiol. 70:133.

(15) Smith, G.V.S. & Smith, O.W. 1938. Observations concerning the metabolism of estrogens in women. Am. J. Obs. and Gynec. 36:769.

(16) Fisch, W.R. & Dorfman, R.E. 1941. The conversion of α-estradiol to estrone by the guinea-pig. J. Biol. Chem. 140:83.

(17) Id. 1942. The conversion of α-estradiol to estrone and B-estradiol by the ovariectomizedhisterectomized rabbit. J. Bio. Chem. 143:15.

(18) Heard, R.D., Bauld, W.S. & Hoffman, M.M. 1941 α-estradiol and progesterone metabolism. J. Bio. Chem. 141:709.

(19) Mather, A. 1940. Proc. Am. Soc. Bio. Chem., J. Biol. Chem.

141:709.

(19) Mather, A. 19401 Proc. Am. Soc. Bio. Chem., G. Biol. Chem. 133, and p. lxlll.

(20) Id. 1942. Distributions of estrogens between inimiscible solvants. J. Biol. Chem. 144:617.

(21) Long, J.L. & Evans, H.M. 1922. The oestrous cycle in the rat. Memoirs of the Univ. of California. Vol. 6.

(22) Emmens, C.W. 1939. Reports on biological standards. Medical Research Council, London.

(23) Pincus, G. & Pearlman, W.H. 1941. Steroid excretion in cancerous & non cancerous persons. Cancer Research 1:970.

(24) Higgins G.M. & Anderson, R.M. 1931. Restoration of the liver of the white rat following partial surgical removal. Arch. of Path. 12:186.

(25) Selye, H. 1941. On the role of the liver in detoxification of steroid hormones and artificial estrogens. J. Pharmacol. & Exp. Med. 71:236.

(26) Brouha, L & Simonnet, H. 1927. Recherches sur la distribution de la folliculine dans l'organisme. Comp. Rend. Soc. Biol. 97:459.

(27) Frank, R.T., Goldberger, M.A., Salmon, U.J. & Friedman, R. 1935 Significance of estrogenic factor in tumors & tissues of the human female. Proc. Soc. Exp. Bio. & Med. 32:1665.

(28) Trancu-Rainer, M. 1931. Elimination des hormones sexuelles par les glandes salivaires. Compt Rend. Soc.

Biol. 106:1001.

(29) Callow, R.K. & Parkes, A.S. 1936. The occurrence of oestrin & progestin in adrenal, testis & hypophysis. J. of Physiol. 87:28p.

(30) Fisher, R.B., Krohn, P.L. & Zuckerman S. 1936. The occurrence of an oestrogenic substance in the sexual skin of monkeys. Biochem. J. 30:2219.

(31) Zuckerman S. 1940. The histogenesis of tissues sensitive to oestrogens. Biol. Rev. 15:251.

(32) Geuther, J.T. 1931. Irradiation of ovaries of guinea pigs & its effect on the oestrous cycle. Am. J. Anat. 48:99.

(33) Benoit, J. 1935. L'Ovaire. Hermann, Paris.

(34) Schmidt, J.G. 1935-36. The excretion of theelin in the urine of guinea pigs with irradiated ovaries. Anat. Rec. 64:255.

(35) Corner, G.W. 1938. The sites of formation of estrogenic substances in the animal body. Physiological Reviews. 18:154.

(36) Allen, E., Francis, B.F., Robertson, L.L., Colgate, C.E. Johnston, G.G., Doisy, E.A., Kountz, W.B., & Gibson, H.V. 1924. The hormone of the ovarian follicle. Anat. Rec. 34:133.

(37) Allen, E., Pratt, J.P. & Doisy, E.A. 1925. The ovarian follicular hormone J.A.M.A85:399.

(38) Mac Corquodale, D.W., Thayer, S.A., & Doisy, E.A. 1936. The isolation of the principal estrogenic substance of liquor folliculi. J. Biol. Chem. 115:435.

(39) Westerfeld, W.W., Thayer, S.A., MacCorquodale,D.W., & Doisy, E.A. 1938. The ketonic estrogen of the sow ovaries. J. Bio. Chem. 126:181.

(40) Laqueur E. 1927. Ueber weibliche hormone im besondern das Menformon. Munchen. Med. Wchuschr., 74:2045.

(41) Doisy, E. 1939. Sex & Internal Secretions. Chap. XIII p. 846. William & Wilkins. Baltimore.

(42) Cunningham, B., May, B., & Gordon, S 1942. Presence of estrogenic hormone (s) in testicular material. Proc. Soc. Exp. Bio. & Med. 41:515.

(43) Beall, D. 1940. The isolation of α-oestradiol & oestrone from horse testes. Biochem. J. 34:1293.

(44) Salmon, U.J. 1939. Desoxycorticosterone is estrogenic in the human female. Proc. Soc. Exp. Bio. & Med. 41:515.

(45) Long C.N.H. 1942. A discussion of the mechanism of action of adrenal cortical hormones. Endocrin. 30:870.

(46) Goat, R.A. 1943. Adrenocortical-like tissue in the ovaries of the adrenalectomized ground squirrel. Endocrin 32:488.

(47) Wyman L.C. 1928. The effect of suprarenal inefficiency on the oestrous cycle in the albino rat. Am. J. Physiol. 85:414.

(48) Moon, H.D. 1937. Effect of adrenocorticotropic hormone on the sexual development of spayed rats. Proc. Soc. Exp. Bio. & Med. 37:36.

(49) Wade, N.J., & Haslewood, L.A. 1941. Effect of removal of ovaries & adrenals on opening of the vagina in the albino rat. Endocrinol. 28:624.

(50) Bourne, G. & Zuckerman S. 1941. The influence of the adrenals on cyclical changes in the accessory reproductive organs of female rats. J. of Endocrin. 2:268.

(51) Gardner, W.U. 1941. Estrogenic effects of adrenal tumors of ovariectomizeddmice. Cancer Research 1:632.

(52) Wooley, G.W., Fekete,E., & Little, C.C. 1943. Gonadectomy and adrenal neoplasms. Science 97:291.

(53) Wintersteiner O. 1942. Chap. XXI, p.327 in Glandular Physiology & Therapy. Am. Med. Assoc. Chicago.

(54) Frank, R.T. 1934. A suggested test for functional cortical tumor. Proc. Soc. Exp. Biol. & Med. 31:1204.

(55) Simpson Levy S., & Joll, C.A. 1938. Feminization in a male adult with carcinoma of the adrenal cortex. Endoctinol. 22:595.

(56) Burrows, Cook, 1937. Biochem. J. 31:950. Isolation of androstadiene from the urine of a man with a malignant tumour of the adrenal cortex.

(57) Allen,E. 1926-27. Hormone content of the placenta & chorionic membranes. Proc. Soc. Exp. Bio. & Med. 24:608.

(58) Allan & Dodds. 1935. Biochem. J. 29:285. Hormones in the urine following oopharectomy during pregnancy.

(59) Probstner,A. 1931. Zur Frage der innersekretorischen Tategkeit der Plazenta. Endokrinol 8:161.

(60) Hart, G.H. & Cole, H.H. 1934. The source of oestrin in the pregnant mare. Am. J. Physiol. 109:320.

(61) Dorfman, R.I. & van Wagenen G. 1941. The sex hormone excretion of adult female & pregnant monkey. Surg. Gynec Olest. 73:545.

(62) Westerfeld, W.W., MacCorquodale, D.W., Thayer, S.A., & Doisy, E.A. 1938. The isolation of theelin from human placenta. J. Bio. Chem. 126:195.

(63) Juffman, M.N., Thayer, S.A., & Doisy, E.A. 1940. The isolation of -dehydrotheelin from human placenta. J. Bio. Chem. 133:567.

(64) Corner, G.W., 1940. The rate of secretion of estrogenic hormones by the ovaries of the monkey Macaca Rhesus. Bull. Johns Hopk. Hosp. 66-67:407.

(65) Zuckerman, S., 1936. The interrelation of oestrone & progestin in the menstrual cycle. J. Physiol. 86:31P.

(66) Hisaw, F., & Greep, R.O. 1938. The inhibition of uterine bleeding by estradiol & progesterone & associated endometrial modifications. Endocrin. 23:1.

(67) Werner, A.A. 1941. A survey of estrogenic dosage. Int. Abst. Lung., 73:49.

(68),Koch,J.C.1937. Cold Spring Harbor Symposia on quantitative

biology Vol. V. The Jacques Cattell Press. Lancaster, Pennsylvania.

(69) Smith, G.V.S, Smith, OW. & Pincus, G. 1938. Total urinary estrogen, estrone & estriol during a menstrual cycle & a pregnancy. Am. J. Physiol. 121:98.

(70) Gustavson, R.G., Mason, L.W., Hays, E.E., Wood, T.R. & D'Amour, F.E. 1938. The quantitative determination of estrogenic substances in normal females during the menstrual cycle. Am. J. Obst. Gynec. 35:115.

(71) Kemp, T. & Pederson-Bjergaard, K. 1937. Absorption & excretion of oestrous by human organisms. Lancet 233:842.

(72) Schiebke, H. 1930. Zentalbe. J. Gynak. 54:1734.

(73) Dohrn, M., & Faure, W. 1928. Ueber die Ausscheidung des weiblichen Sexualhormons. Klue. Wchnschr. 7:943.

(74) Oesting, R.B. & Webster, B. 1938. The sex hormone excretion of children. Endocrine. 22:307.

(75) Saunders, F.J., & Cole, H.H. 1936. Age and the qualitative ovarian response of the immature rat to mare gonadotropic hormone. Proc. Soc. Exp. Biol. & Med. 33:504.

(76) Dingemanse, E., Borchardt, H., & Laqueur, L.E. 1937. Capon comb-growth promoting substances (Male hormones in human urine of males and females of varying ages. Biochem. 2.31:500.

(77) Pearlman, W.H. & Pincus, G. 1943. The metabolism of estrone in man. J. Biol. Chem. 147:379.

(78) Doisy, E. 1942. Biol. Symp. Vol. IX p. 21. The Jacques Cattell Press. Lancaster, Pa.

(79) Marker, R.E. 1938. The origin and interrelationship of the steroidal hormones. J. Am. Chem. Soc. 60:1725.

(80) Palmer A. 1940. The excretion of free estrogen during uterine bleeding. J. Endocrin. 2:70.

(81) Hain, A.M. 1940. The excretion of estrogen & pregnandiol by pregnant and parturient women: normal & toxaemic cases. J. Endocrin. 2:104.

(82) Sainton, P., Simonnet, H., & Brouha, L. 1937. Endocrinologic clinique, therapeutique et experimentale. Masson, Paris.

(83) Pincus, G. & Pearlman, W,H. 1942. Metabolism of estrone in man & non pregnant women. Endocrin. 31:507.

(84) Allen, E., Diddle, A.W., Burford, T.H. & Elder, J.H. 1936. Analyses of urine of the chimpanzee for estrogenic content during various stages of the menstrual cycle. Endocrin. 20:546.

(85) Fish, W.R., Young, W.C. & Dorfman, R.I. 1941. Excretion of estrogenic & androgenic substances by female & male chimpanzees. Endocrin. 28:585.

(86) Allen, E., Maddux, W.P. & Kennedy, J. W. 1930. Ovarian & anterior pituitary hormone from the pregnant monkey. Proc. Soc. Exp. Boi. & Med. 28:403.

(87) Cook, J.W., Dodds, E.C., Hewett, C.L. & Lawson, W. 1934.

The oestrogenic activity of some condensed ring compounds in relation to their other biological activities. Proc. Roy. Soc. B. 114:272.

(88) Cook, J.W., Dodds, E.C. & Greenwood, A.W. 1934. Sex change in the plumage of Brown Leghorn Capons following the injection of certain synthetic oestrus producing compounds. Proc. Roy. Soc. B. 114:286.

(89) Janney, C.J. & Walker, B.S. 1930. Estrogenic substances. An apparatus & methods for preparation of stable extracts from natural sources. Endocrin. 14:101.

(90) The rat in laboratory investigation. Griffith, J.O. Editor. Chap. 1. J.P. Lippicot Comp. Philadelphia 1943.

(91) Zondek, B. 1937, Oestrogenic substances in the Dead Sea. Nature, 140:240.

(92) Aschheim, S., & Hohlweg, W. 1933. Ueber das Vorkommen oestrogener Wirkstoffe in Bitumen. Dent. Med. Wochschr. 59:12.

(93) Butenandt, A., & Jacobi, H. 1933. Ueber die Darstellung eines kristallisierten pflanflichen tokokinius (Thelykinins) und seine identifizierung mit dem α-Follikelhormon. Z. Physiol. Chem. 218:104.

(94) Skarzynski, B. 1933, An oestrogenic substance from plant material. Nature, 131:766.

(95) Steidle, H. & Banor, E.E. 1932. Ueger weibliche Sexualhormone bei einzelligen Tieren. Naunyn--Schmeidelberg's Archif 163:

(96) Steidle, H. 1930. Naunyn - Schmiedelbergs Archif: Ueber die Verbreitung des weiblichen Sexualhormons. 157:89.

(97) Thomsen, E. 1940. The relation between corpus allatum & ovaries in adult flies. Nature 145:28.

(98) Donahue, Y.K., DeWitt Jennings, E. 1937. The occurrence of oestrogenic substances in the ovaries of cohinoderms. Endocrinology 21:690.

(99) Loewe, S., Romdenfusch, W., Voss, H.E. & Von Heurn, W.C. 1932. Nachweis-des Sexualhormon-Vorkommens bei Schmetterlingen. Biochem. Ztschrft. 244:347.

(100) Delage, Y. 1884. Evolution de la Sacculine. Arch. Zool. Exprim. (Serie II) t. 1

(101) Smith, G. 1906. Rhizocephala,In Fauna und Flora des Golfes von Neapel. Monog. 29.

(102) Gorbman, A. 1941. Comparative anatomy and physiology of the anterior pituitary. Quart. Rev. Biol. 16:294.

(103) Lutz, B.R., Wyman, L.C. 1932. The evolution of a carotid sinus reflex and the origin of vagal tone. Science 75:590.

(104) Hanssler, E.P. 1934. Ueber das Vorkommen von -Follikelhormon im Gengsturin Helv. clinic. acta, 17:531.

(105) Cartland, R.G., Meyer, R.K., Miller, L.C., & Marshall, H.R. 1935. A comparison of theelin prepared from stallion urine human urine, and from theelol, with notes on the colorimetric estimation of theelin & theelol. J. Biol. Chem. 109:213.

(106) Beerstecher, E,, 1942. Variation in estrogen content of urine in female rabbits. Endocrin. 31:26.

(107) D'Amour, F.E., Funk, D. & Glendenning, M.B. 1936. Presence of estrin in rat pregnancy urine. Proc. Soc. Exp. Bio. & Med. 35:26.

(108) Dorfman, R.I. 1937. The excretion & metabolism of theelin in the sexually immature rat. J. Bio. Chem. 119:XXV.

(109) Marker, R.E. 1939. Ketonic steroids from cow's pregnancy urine & bull's urine. J.and Proc. Am. Chem. Soc. 61:944.

(110) Longwell, B.B. & McKee, F.S. 1942. The excretion of estrogens in the bile and urine after the administration of estrone. J. Bio. Chem. 142:757.

(111) Pincus, G. & Pearlman, W.H. 1942. Metabolism of estrone in man & non pregnant women. Endocrin. 31:507.

(112) Heard, R.D.H. & Hoffman, M.M. 1941. The fate in man of injected α -estradiol. J. Bio. Chem. 141:329.

(113) Schiller, J. & Pincus, G. 1943. The fate of -estradiol and of estriol injected into a human male subject. Arch. Biochem. 2:317.

(114) Kemp, T. & Pedersen - Bjergaard, K. 1937. Absorption & excretion of estrone by the human organism. Lancet. 233:842.

(115) Mazer, C. & Israel, S.L. 1937. Studies on the optimal dosage of estrogens: an experiment & clinical evaluation. J. A. M. A. 108:163.

Baerstecher, E. 1942.

(116) Frank, R.T., Goldberger, M.A., & Spielman F. 1931. Utilization and excretion of female sex hormone. Proc. Soc. Exp. Biol. & Med. 29:1229.

(117) Westerfeld, W.W., & Doisy, E.A. 1937. Ketonic & non ketonic estrogens. Ann. Int. Med. 11:267.

(118) Marker, R.E. & Hartman, C.H. 1940. Assays of urine from rhesus monkey for pregnandiol & other steroids. J.,Biol. Chem. 133:529.

(119) Doisy, E.A., Thayer, S.A., & Van Bruggen 1942 in Federation Proc. Vol. 1 p. 202.

(120) Van Bruggen as cited by Doisy in Biological Symposia Vol.IX p. 30. 1942. The Jacques Cattell Press. Lancaster, Pa.

(121) Kunde, M.M., D'Amour, F.E., Carlson, A.J., & Gustavson, R.J. 1930. The effect of estrin injections on the basal metabolism, uterine endometrium, lactation, mating and maternal instincts in the adult dog. Am. J. Physiol. 95:630.

(122) Stamler, C.M., 1937. The fate of folliculine in the dog. Bull. Biol. et Med. Experim. III:31.

(123) Dingemanse, E. & Tyslowitz, R. 1941. Urinary elimination of estrogens injected in dogs. Endoctin. 28:450.

(124) Cantarow, A., Rakoff, A.E., Paschkis, K.E., & Hansen, L.P. 1942. Excretion of estrogens in bile. Proc. Soc. Exp. Biol. & Med. 49:707.

(125) Smith, G.V., & Smith, W.O. 1931. Studies on the urinary excretion of oestrin, with special reference to the effect of the luteinizing hormone & progestin. Am. J. Physiol. 98:578.

(126) Pincus, G. & Zahl, P.A. 1937. The fate of estrins injected into the rabbit. J. Gen Physiol. 20:879.

(127) Stroud, L.W. 1939. Recovery of injected oestrogenic substances from rabbit urine. J. of Endocrin 1:201.

(128) Fish, W.R. & Dorfman, R.I. 1940. Comversion of estradiol to estrone *in vivo* Science. 91:338.

(129) Fish, W.R. & Dorfman, R.I. 1941. The metabolism of -estradiol to estrone. J. Biol. Chem. XI:140.

(130) Emmens, C.W. 1943. The urinary excretion of estrogens following the injection of pro-oestrogens in the guinea-pig. J. of Endocrin. 3:316.

(131) Zondek, B. 1934. The fate of follicular hormone in the living body. Lancet II:356.

(132) Dingemanse, E., & Laqueur, E. 1937. On the inactivation of estrone, estradiol & their monobenzoates in the organism. Am. J. Obst. Gynec. 33:1000.

(133) Golden, J.B., & Severinghaus, E.L. 1938. Inactivation of estrogenic hormone of the ovary by the liver. Proc. Soc. Exp. Biol. & Med. 39:361.

(134) Evans, H.M. & Buhr, G.O. 1925. Increased efficacity of subcutaneous when compared with intraperitoneal administration of the ovarian hormone. Am. J. Physiol. 77:518.

(1) Allen, E

(135) Fee,A.R.,Marrian, G.F.& Parkes,A.S.1929. The significance of the occurrence of estrin in male urine.J. of Physiol. 67:377.

(136) Silberstein,F.,Engel, P.& Molnar,K.1933.Ueber das Auftreten eines Brunstoffes in Blut und geweben under Pathologischen Verbaltuisse.Klinische Wchschrft. 12:1693.

(137) Talbot,N.B.1939. The inactivation of endogenous estrogens by the liver. Endocrin. 25:60.

(138) Pincus,G.& Martin,D.W.1940. Liver damage & estrogen inactivation. Endocrin. 27:838.

(139) Zondek,B.& Sclow,J.1941. Inactivation of estrone by liver after exclusion of the reticulo-endothelial system. Proc. Soc. Exp. Biol. & Med.46:276.

(140) Biskind,G.R.& Mark,J.1939. The inactivation of testosterone propionate &estrone in rats.Bull. Johns Hopk. Hosp. 65:212.

(141) Biskind,G.R.1941. The inactivation of estradiol & estradiol benzoate in castrate female rats. Endocrin. 28:894.

(142) Segaloff,A.& Nelson,W.O.1941. Absorption & metabolism of estrogen.Estrogen assay by intrasplenic injection. Proc.Soc.Exp. Biol.& Med. 48:33.

(143) Glass,S.J.,Edmunson,H.A.,& Soll,S.N. 1940. Sex-hormone changes associated with liver disease. Endocrin. 27:749.

(144) Israel,S.L.,Meranze,D.R.& Johnston,C.G. 1937. The inactivation of estrogen by the liver.Am.J. Med.Sci. 194:835.

(145) Heller,C.G.1940. The effect of liver & uterus upon estrone,estradiol & estriol.Endocrin.26:619.

(146) Heller,C.G.& Heller,E.J.1943. Metabolism of estrogens: effect of frequency upon metabolism in vitro of estrone,estradiol & estriol.Endocrin.32:64.

(147) Twombly,G.H.& Taylor,H.C.1942. Inactivation & conversion of estrogens in vitro by liver & other tissues from human cancer patients & from mice of strains susceptible to mammary carcinoma. Cancer Research.2:811.

(148) Cantarow,A.,Rakoff,A.E.,Paschkis,K.E.,Hansen,L.P. & Walking,A.A.1942. Excretion of estrogen in bile. Endocrin.31:309.

(149) Cantarow,A.,Paschkis,K.E.,Rakoff,A.E.,&Hansen,L.P. 1943. Studies on inactivation of estradiol by the liver. Endocrin.33:309.

(150) Segaloff,A.1943. The intrasplenic injection of estrogen & their esters. Endocrin.33:209.

(151) Engel,P. & Navratil,E.1937. Beitrag zur Zerstorung des Follikelhormons bei Kaltbluter.Biochem. Ztschrft.292:434.

(152) Engel,P.1941. Inactivation of estrogens by the liver of guinea-pigs in different sexual states. Endocrin. 29:290.

(153) Euler,H.V.& Zondek,B.1934. Follikulin und Pflanzen. Biochem.Ztschrft.271:64.

(154) Westerfeld,W.W.1940. The inactivation of oestrone. Biochem.J.34:51.

(155) Graubard,M.& Pincus,G.1942. Steroid metabolism:estrogens and phenolases. Endocrin.30:265.

(156) Biskind,M.S. & Shelesnyak,M.C.1942. Effect of vitamin B complex deficiency on inactivation of ovarian estrogen in the liver. Endocrin.30:819.

(157) Biskind,M.S. & Biskind,G.R.1942. Effect of vitamin B complex deficiency on inactivation of estrone in the liver. Endocrin.31:109.

(158) Thorn,G.W. & Engle,L.L. 1938. The effect of sex hormones on the renal excretion of electrolytes. J.Exp. Med.68:299.

(159) Janes,R.G.& Nelson,W.O.1942. The influence of diethylstilbestrol on carbohydrate metabolism in normal & castrated rats. Am.J.Physiol.136:136.

(160) Ingle,D.J.& Kendall,E.C.1937. Atrophy of the adrenal cortex by the administration of large amounts of cortin. Science 86:245.

(161) Beall,D. 1939. The isolation of oestrone from the adrenal gland. Nature.144:76.

(162) Cole,V.V.& Harned,B.K.1942. Adrenal & pituitary weights in rats with reduced glucose tolerance.Endocrin. 30:146.

(163) Hall,K.& Korenschevsky,K.1938. Effects of castration & of sexual hormones on the adrenals of male rats. J. Physiol. 91:365.

(164) Hashimoto,E.J. 1940. The effect of the duration ofthe post-operative interval on the quantitative changes in adrenal glands of ovariectomized albino rats. Anat. Rec. 81:205.

(165) Ingle,D.J. 1938. The effects of administering large amounts of cortin on the adrenal cortices of normal & hypophysectomized rats. Am.J.Physiol.124:369.

(166) Selye,H.,Collip,J.B. & Thompson,D.L.1935. Effect of oestrin on ovaries & adrenals.Proc.Soc.Exp.Biol. & Med. 32:1377.

(167) Bourne,G.& Zuckerman,S.1941. Changes in the adrenals in relation to normal & artificial threshold oestrous cycle in the rat. J.Endocrin. 2:283.

(168) Golla,M.L.& Reiss,M. 1941. Observations on adrenocorticotrofluic action. J. Physiol. 100:1P.

(169) Shumaker,H.B.& Firor,W.M. 1934. The interrelationship of the adrenal cortex & the anterior lobe of the hypophysis. Endocrin. 18:676.

(170) Grollman,A. 1936. The adrenals. Chap.XIV. Williams & Wilkins. Baltimore

(171) Astwood,E.B. 1939. Changes in the weight & water content of the uterus of the normal adult rat. AM.J.Physiol. 126:162.

(172) Thorn,G.W.& Harrop,G.A.1937. The "sodium retaining effect "of the hormones. Science,86:40.

(173) Everett,J.W.1940. The restoration of ovulatory cycles & corpus luteum formation in persistent estrous rats by progesterone. Endocrin. 27:681.

(174) Pfeiffner, C.A. 1941. Hormone factors in the formation & maintenance of corpus luteum in "constant"estrous rats, Anat. Rec.79:50. Abst.#122

(175) Witschi,E.& Pfeiffner.C.A. 1935. The hormonal control of oestrus,ovulation & mating in the female rat. Anat. Rec. 64:85.

(176) Parker,G.H.1932. Humoral agents in nervous activity with special reference to chromatophores. Cambridge Unit. Press.

(177) Zuckerman,S.,Palmer,A.,& Bourne,G.1939. Changes in the water-content of organs and tissues as result of stimulation by oestradiol. Nature.143:521.

(178) Thayer,S.A.,MacCorquodale,D.W. & Doisy,E.A.1938. The ketonic estrogen of sow ovaries. J.Biol. Chem. 126:181.

(179) Pearlman,W.H. & Pearlman,M.R.J. 1944. A differential analysis of mixtures of -and -estradiol in urine extracts. Arch. Biochem.4:97.

(180) Brues,A.M.,Drury,D.R.& Brues,M.C.1936. A quantitative study of cell growth in regenerating liver. Arch. Path. 22:658.

(181) Emmens,C.W. 1941. Precursors of oestrogens. J.Endocrin. 2; 444.

(182) Allen,E.,Hisaw,F.L. & Gardner,W.U.1939. Chap VIII in Sex and internal Secretions.Williams& Wilkins, Baltimore.

(183) Allen,E. 1942. Physiology of the ovaries in Glandular Physiology & Therapy. Am.Med.Assoc.Chicago.

(184) Robson,J.M. 1940. Recent advances in Sex and reproductive physiology. The Blakiston Comp.Philadelphia.

(185) Smith,P.E.1927. The disabilities caused by hypophysectomy and their repair in the rat.J.A.M.A.88:158.

(186) Smith,P.E.1930. Hypophysectomy and a replacement therapy in the rat. Am.J.Anat. 45:205.

(187) Hisaw,F.L.& Leonard,S.S.1930. Relation of the follicular and corpus luteum hormones in the production of projestational proliferation of the rabbit uterus. Am. J. Physiol.92:574.

(188) Hisaw,F.L. 1938. Hormones regulating the development of the female reproductive tract of macacus rhesus monkey.Colloque International. Herman. Paris.

(189) Selye,H. & Clarke,E. 1942. The action of steroid compounds on the vaginal epithelium of the rat. Am. J. Med. Sci. 204:401.

(190) Courrier,R.1930. Recherches sur l'antagonisme des hormones folliculaires et luteiniques. Paris Comp. Rend. Soc. Biol. 104:280.

(191) Courrier,R. 1938. Colloque International. Herman.Paris.

(192) Dorfman,R.I.,Greulich,W.W. and Solomon,D.I.1937. The excretion of androgenic and estrogenic substances in the urine of children. Endocrin.21:741.

(193) Robson,J.M.1936-37. The action of progesterone on the uterus of the rabbit and its antagonism by oestrone. J. Physiol.88:100.

(194) Robson, J.M.1937. Maintenance by oestrin of the luteal function in hypophysectomized rabbit. J.Physiol. 90:435.

(195) Robson, J.M. 1940.Prolongation of pregnancy in the hypophysectomized rabbit by progesterone and oestrogens. J.Physiol.97:517.

(196) Allen,E.1938.Growth of tissues in response to ovarian follicular hormone and associated experiments. Colloque International. Herman.Paris.

(197) Lwoff,A. 1925. Influence d'extrait de glandes et d'organes sur la vitesse de multiplication des Infusoires. Paris Comp. Rend.Soc.Biol.93:1352.

(198) Rahn,H. 1939. The corpus luteum of reptiles. Anat. Rec. 72:supp.55.

(199) Leblond,C.P. & Noble,G.K.1937. Prolactin-like reaction produced by hypophysis of various vertebrates. Proc.Soc.Exp.Biol. and Med.36:517.

(200) Hill,R.T. & Strong,M.T. 1938. Ovaries secrete male hormone. Endocrin.22:663.

(201) Leonard,L.L.,Hisaw,F.L. and Ferold,H.L. 1932. Further studies of the follicular-corpus luteum hormone relationship in the rabbit.Am.J.Physiol. 100:111.

(202) Reichstein,T. and Shoppee,C.W.1943. The hormone of the adrenal cortex in vitamins & hormones. N.Y. Acad. Press.

(203) Butenandt,A.1936. Zur Physiologie des Androstenediols-(3, 17). Naturwissenschaften 24:15.

(204) Butenandt,A.1937. Recherches chimiques sur la specificite physiologique des hormones genitales. Bull. Soc. Chim. Biol. 19:1477.

(205) Zuckerman,S.1936. The interrelation of oestrone and progestin in the menstrual cycle.J.Physiol.86:31.

(206) Kendall,E.C.1942. Hormones of the adrenal cortex. Endocrin. 30:853.

(207) Corey,E.L. and Britton,S.W. 1933. The estrous cycle and the adrenal glands.Proc.Soc.Exp.Biol.and Med. 30:592.

(208) Id. 1931. The induction of precocious sexual maturity by cortico-adrenal extract.Am.J.Physiol.99:33.

(209) Gutkelch,A.N. & Zuckerman,S.1937. The red cell count of macaques in relation to the menstrual cycle. J. Physiol. 91:269.

(210) Aykroyd,D.E. & Zuckerman,S.1938. Factors in sexual skin oedema. J.Physiol.94:13

(211) Eversole,W.J.,Edelman,A. & Gaunt,R.1940. Effect of adrenal cortical transplantations on life maintenance and water intoxication.Anat.Rec.76:271.

(212) Id. 1943. Methods of administering desoxycorticosterone and the problem of its inactivation by the liver. Endocrin.32:51.

(213) Burrill,M.W. and Greene,R.R. 1942. Capacity of rat's liver to inactivate desoxycorticosterone acetate. Endocrin. 31: 582.

(214) Mark,J. 1942. Inactivation of desoxycorticosterone acetate. Endocrin. 31:582.

(215) Burrill,M.W. & Greene,R.R.1941. The liver and the adrenal androgen of the rat. Endocrin. 28:874.

(216) Id. 1942. Effect of rat's liver on activity of testosterone and methyl testosterone.Endocrin.31:73.

The Metabolism of Estrogen.

Abstract.

The fondamental discoveries of Allen and Doisy, of Aschheim and Zondek and the availability of chemically pure estrogens have permitted an intelligent investigation of the metabolism of estrogens. The most definite findings were the inactivation of estrogens by the body to an amount exceeding 80 per cent of the injected substance in any animal species and the conversion from one estrogen to another. Unexpected was the fact that no relationship could be established between the type of conversion and the zoological classification of any given species; also that in all investigated species, the secondary sex organs which _a priori_ could be expected to play the major part as sites of estrogen metabolism seem to be entirely inactive. The bulk of experimental evidence points to the liver as being the principal organ in estrogen inactivation. Since this type of experiment as well as that based on recovery of urinary estrogens gave only end results, it was considered worthy to investigate some aspects of the intermediary steps of both anabolism and catabolism of estrogens.

Concerning the anabolism of estrogens the role of the adrenal glands was investigated and it was found that under adrenocorticotrophic stimulation the estrogen output of adult female rats was increased as detected by the vaginal smear test. This occured best in partially hepatectomized

animals,ovariectomized or not,while ovariectomized controls were unaffected. This fact would indicate that the normal liver inactivates the estrogen produced by the adrenal glands as all previous similar experiments devised in order to see the fate of estrogens passing through the liver have shown.However, this was contradicted by other experiments in which the estrous cycles were not disturbed in rats with the adrenals transplanted into the spleen. Since the adrenal hormones responsible for maintenance of life,of blood sugar level and liver glycogen stores were not inactivated either,it was concluded that the liver behaves differently with respect to endogenous steroid hormones,which are not inactivated,and to exogenous steroids,which usually are inactivated.

Precise data on the role played by the liver and the catabolism of exogenous estrogens was shown by two groups of experiments performed at the "body-level"and the "organ- level".

The injection of estrone into normal and partially hepatectomized male rats showed that this estrogen before being inactivated is partially converted to both estradiol and estriol. Also that there is a quantitative relationship between the amount of liver tissue present and the rate and intensity not only of the expected inactivation but of the conversion as well: the less liver tissue present the less conversion occurs and the more delayed the inactivation.

Thus,for the first time it was definitely established

that conversion of estrogens is a function of the liver. It is also noteworthy that the direction of conversion occurs not only towards the less potent estrogen, estriol, but also to the more potent estradiol.

In order to understand better the role of the liver, as well as that of other organs, in estrogen metabolism, perfusion experiments *in vitro* on female rat livers were performed. Factors such as condition of the animal furnishing the organ, amount and nature of the estrogen used and perfusion time were strictly considered. The conclusions reached as a result of the preceding experiments were entirely confirmed since conversion of estrone to both estradiol and estriol were found again. In addition several new facts were established. Estradiol is converted by rat liver to estrone and estriol. The rate of conversion and inactivation is higher than for estrone thus establishing that estradiol is a more labile estrogen than estrone. In contrast to these two estrogens, estriol is not convertible to any known estrogenic substances and best resists inactivation by the liver. It is also suggested that conversion to estrone and estriol is not the only possibility and that other metabolites can be produced either between the conversion from estradiol to estrone or after the conversion to estriol. The nature of such intermediary products awaits further chemical identification. It is also probable that new experiments at the "cell-level"

will have to be devised.

The perfusion of rat kidney with estradiol showed that this organ also is concerned with conversion and inactivation. However, as compared with the liver the direction of conversion is qualitatively and quantitatively different since no estriol is produced and the rate of conversion and inactivation is slower.

Not all organs, however, have such abilities as shown by the fact that estradiol perfused through a rat's heart is not affected.

It is interesting to compare the catabolism of exogenous estrogens in the rat to that in the human species. Since the direction is the same in both, this may account for the suitability of the rat for bioassay in clinical investigations.

The differential behaviour of the rat liver with respect to exogenous and endogenous estrogens as indicated by the experiment on the transplanted adrenals was confirmed in perfusion experiments. Three hours will suffice for the perfused liver to inactivate 2000 rat.units of estradiol, while in the same time the perfusion of the liver of a female rat in pro-estrus will leave unaffected the 15 rat units which this liver contained at the beginning of the experiment. The same result was obtained after a five hour perfusion. The amount of the endogenous estrogen stored by the liver of a female rat in pro-estrus was established by direct extraction of

estrogenic substances from the organ. Since previous experiments have shown that as a result of the passage of ovarian estrogens through the liver the vaginal smear changes to the diestrous type, it must be concluded that the liver acts as a threshold organ releasing the stored endogenous estrogens into the systemic circulation when a definite level has been reached. It is probable that this threshold is subjected to variations in accordance with the estrous cycle. It is not possible as yet to establish the link with other regulatory factors.

Concerning the nature of endogenous estrogens our investigations were limited to the adult male rat. It was found that this animal excretes estradiol and estrone in the urine. Since no experiments were conducted in order to establish the source of these two estrogens, it is suggested that, in accordance with the findings in other species, that they are produced by the testes and/or adrenals.

It is interesting to consider that an organ like the liver, which is involved in the least specialized types of metabolism, is also responsible for the metabolism of estrogens. This is one more proof that specificity is not too narrow. It is certain that a new function can be ascribed to the liver, namely that of converting and inactivating estrogen. More striking is the fact that potentiation is also possible, as shown by conversion from estrone to the more potent estradiol and from

the inactive 4,4'dihydroxy-diphenyl to a potent estrogen.

It is generally accepted that the regulatory mechanism of estrogen metabolism is the resultant of the interaction of several factors, the pituitary-gonad system, the synergistic and/or antagonistic reactions of various steroids as related to each other, and effector organ receptivity. As a result of our investigations, we conclude that the liver plays a large part in the metabolism of estrogen and has to be included, together with the previously mentioned factors, in a unified system of regulatory mechanisms.

Born in Bucharest,December 30,1905. The parents being Adolphe and Yvette. Went to Lycée and obtained the B.A.,degree at the University of Paris in 1927,the M.D., degree at the Faculty of Medicine of Paris in 1935, Worked at the Hôpital de la Pitié and the Hospital of St.Louis in Paris. Obtained the degree of Licencié ès Sciences at the Sorbonne in 1936. Worked at the Institut Oceanographique of Monaco,1937-38. In 1941 went to Equatorial Africa and worked on problems of applied Parasitology. After 6 months came to this country. In 1942 had a fellowship at the Clark University. Since,has published several scientific papers and became a member of the American Society for the Study of Internal Secretions.

BOSTON UNIVERSITY
1 1719 02481 4743